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Effects of Soil Insecticides on Apple Trees and Resulting Effect on Mite Nutrition[1]

J. G. Rodriguez, H. H. Chen, and Walter T. Smith, Jr.,[2] *University of Kentucky, Lexington*

Abstract

Apple trees were grown in semifield conditions in cans of soil treated with several levels of DDT, dieldrin, and BHC. Fertilizer was added to the soil in 1956 but not in 1957. Females of *Panonychus ulmi* (Koch) and *Tetranychus telarius* (L.) were cultured on leaves detached from the trees and the resulting progeny were counted. Reactions of the trees were measured. Analyses of foliage were made for total nitrogen, phosphorus, and potassium. Analyses of variance were performed on the tree reactions, the results of the foliage analyses, and the mite counts. Tests for correlation coefficient (r) were made on absorbed elements with populations.

Root weight was lowered by all treatments in 1956, significantly by dieldrin. DDT at 100 pounds per acre in 1957 increased root weight, not significantly. BHC and dieldrin depressed twig growth, not significantly.

Changes in chemical composition of the slower growing, woody apple trees were less striking than those previously found in beans, soybeans, and cotton. On fertilized soil in 1956 DDT at 100 pounds per acre elevated absorbed phosphorus to 0.32% and depressed populations of both species, and at 1,000 pounds increased both populations over the untreated check and significantly over the 100 pound level. In 1957 DDT at 100 and 4,000 pounds on unfertilized soil significantly increased per cent nitrogen over the untreated check and also increased the populations. That year the absorbed phosphorus generally fell below 0.20% and it was significantly correlated positively with *T. telarius* populations while the relationship between the two in the previous year was generally negative. These results support previous findings of a similar nature concerning ranges of absorbed phosphorus and *T. telarius* populations.

Dieldrin at 50 pounds per acre increased *T. telarius* over the check and at 500 pounds depressed both species below the check and 50-pound levels. BHC depressed *T. telarius* populations, only, at both 20- and 200-pound levels.

Thus, DDT at 100 pounds per acre has been shown to be capable of significantly affecting both the chemical composition of the apple tree and the mite populations developing thereon. This amount, and even more, of DDT could easily be applied to a commercial orchard over two seasons in a normal spray schedule. Thus, the buildup of insecticide residues on the orchard floor should be considered a potential factor influencing the mite populations. Also evident in this work was the fact that insecticide effects on both foliage composition and mites may vary with the fertility level of the soil.

In the first paper of this series, the general subject of soil insecticide effect on plants was discussed and the existing literature reviewed concerning their absorption and translocation in the plant, persistence in the soil, effect on specific plants or plant parts, effects on soil microorganisms and effects on plant composition (Rodriguez *et al.* 1957). In this report the fact was established that the addition of such materials as aldrin, dieldrin, chlordane, lindane, BHC, and DDT affected the nitrogen, phosphorus, and potassium composition of Black Valentine beans, soybeans, and cotton and also the populations of mites developing on these host plants. Concurrently with these studies, the comparative nutrition of the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), on apple trees in nutrient culture was being investigated; in this work the fact was established that host plant nutrition is an important consideration in the understanding of mite population development in the orchard. When nitrogen supply was increased from 20 to 200 p.p.m., from 2× to 10× as many *T. telarius* resulted and from 1.4× to 1.8× as many *P. ulmi* resulted during the course of the test periods of 8 and 10 days, respectively. During one season a further increase in mites of both species occurred when the nitrogen supply was further increased to 800 p.p.m. (Rodriguez 1958). Breukel & Post (1959) have recently reported that the population density of *Metatetranychus* (*Panonychus*) *ulmi* increased in neglected apple orchards after pruning and manuring with ammonium sulphate and without pest control, concurrently with an increase in nitrogen content of apple leaves. In a well kept orchard with a rather high population of mites, the population and nitrogen content of the leaves decreased after pruning and manuring were stopped. Under laboratory and semifield conditions the reproduction rate of the mite on high-nitrogen leaves was 1.6 to 1.7 times that on low-nitrogen leaves, thus confirming their field observations.

The problem of insecticides in the soil of orchards is a

[1] This study was sponsored in part by a grant-in-aid from the Rockefeller Foundation and is the second in a series of papers dealing with the general subject of the effects of soil insecticides on plants and mite nutrition.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

Partial cost of publication of this paper was met by the Department of Entomology and Botany, Kentucky Agricultural Experiment Station. Accepted for publication March 18, 1960.

[2] Associate Professor of Entomology, former graduate student, and Professor of Chemistry, respectively.

realistic one. Arsenic-toxic soils have been studied by Overley (1950) in Washington and cumulative spray residues in soil by Gould and Hamstead in West Virginia (1951). Spray schedules include numerous cover sprays during the season and accumulation of insecticides on the orchard floor is an inevitable result. Moreover, at least one chlorinated hydrocarbon is now being applied to orchard floors to control mice. To be sure, mite populations build up by direct effect of the chemical on the predators. It is well known that predators of orchard mites are killed by DDT and other chlorinated hydrocarbons. Recently Hurlbutt (1958) studied the mite fauna in apple orchards. Sampling the orchard floor, he found that Tetranychidae were more numerous in heavily sprayed orchards and the number of predatory species was smaller than in abandoned or lightly sprayed orchards. There is evidence also that soil insecticides affect plants in orchards. Continuous lead arsenate sprays in apple orchards have resulted in the surface soil becoming toxic to many kinds of cover crops, especially legumes (Overley 1950). Klostermeyer & Skotland (1959) have reported that hops die out as a result of heptachlor toxicity in the soil. An accumulation of insecticides in the soil sufficient to exert phytotoxic effects would no doubt bring about altered plant nutrition in the cover crops and perhaps even in the apple trees growing in the same soil. Fleschner (1958) has observed a stimulative effect of DDT sprays on mite reproduction on citrus trees; laboratory experiments indicated that this effect was partly owing to induced physiological changes in the host plant.

Thus, it was deemed advisable to investigate possible effects of soil insecticides on the NPK nutrition of apple trees and the nutrition of *P. ulmi* (Koch) and *T. telarius* (L.) developing thereon. The present paper deals with the results of these semifield experiments during two seasons.

EXPERIMENTAL.—*Tree Culture.*—Carefully selected Close apple trees approximately three-quarters of an inch in diameter which were trimmed to a whip about three feet long and the roots of which were also trimmed as evenly as possible were set in early April each year in 5-gallon capacity cans filled with screened Maury silt loam. A granulated, balanced fertilizer, 10-10-10, was added at the rate of 500 pounds per acre to the soil in 1956. None was added in 1957. The fertilizer and insecticide treatments were calculated on the basis that 6 inches of the average soil over the surface of 1 acre equals 2 million pounds. The insecticides were added as wettable powders in two doses; the first half was added when the trees were planted, the remainder a month later. Both applications were worked into the top 2 inches of soil. The insecticides included DDT during both seasons with dieldrin in 1956 and BHC in 1957. Six treatments including an untreated check, each replicated three times, were used in 1956 and five treatments in 1957. The dosages of insecticides expressed as pounds per acre during the two seasons are shown in table 1.

Mite Technique.—The mite tests were conducted from late July to early September. The technique for detached leaf culture using apple leaves has been described in the earlier paper dealing with apple trees (Rodriguez 1958). In 1956, four experiments using *P. ulmi* and two using *T. telarius* were completed; in 1957 there were six and four experiments, respectively.

Table 1.—Reactions of apple trees treated with soil insecticides.

Treatment	Lb./A.	Twig Growth in Mean Feet/Replicate		Dry Weight of Roots in Mean Grams/Replicate	
		1956	1957	1956[a]	1957[b]
Untreated		3.08	3.17	61.3	136.5
DDT	100	2.76	3.24	51.7	179.6
	1,000	2.62	—	53.7	—
	2,500	2.65	—	49.0	—
	4,000	—	3.66	—	137.2
Dieldrin	50	3.32	—	36.6[c]	—
	500	2.96	—	38.3[c]	—
BHC	20	—	3.15	—	140.7
	200	—	2.85	—	130.8
L.S.D. at 5% level		.69	1.36	15.6	112.0

[a] Weight excluding tap root.
[b] Weight including tap root.
[c] Differs significantly from untreated check.

Tree Reactions.—At the termination of the experiment the trees were washed clean of soil. Total weights of the trees were taken in 1957. In both years the following procedure was pursued; the clean roots were thoroughly air-dried and weighed to determine total root weight and the four longest branches on each tree were cut off the trunk and measured to determine relative tree growth.

ANALYSES.—*Foliage.*—Foliage samples were taken from each tree from approximately the same position on the twig as the leaves used for detached leaf culture. Samplings made during August and early September were combined into a composite sample of foliage from each tree which was analyzed for total nitrogen, phosphorus, and potassium.

Statistical.—Analyses of variance, single classification, were performed to determine the effect of insecticide treatment on tree weight, twig length, root weight, and percentages of nitrogen, phosphorus, and potassium. On the mite populations multiple classification was used to determine the effect of treatment, experiment (series) and the interaction of treatment by experiment on each species. The test for correlation coefficient (r) was performed on the percentage of each element with the total mites of each species that developed on the leaf disks from each replicate; a separate test was made for each insecticide.

RESULTS AND DISCUSSION.—Because of the relatively high level of absorbed NPK evident in the foliage the first year, it was decided that the 1957 set of trees would not be fertilized. Good growth was apparent both years. Because of differences in fertility level of the soil and in the season, a comparison of one year's experiment with the other cannot be made.

Dieldrin and BHC tended to affect twig growth adversely but this reaction did not attain significance either year. Root weight was lowered by all treatments in 1956,

Table 2.—Average per cent absorption of elements in foliage of apple trees grown in fertilized soil and treated with soil insecticides and resulting mite development. 1956.

Insecticide (Lb./A.)	Per Cent Absorbed Element			Average Mites per Leaf Disk	
	N	P	K	*P. ulmi*	*T. telarius*
Untreated	2.50	0.28	2.07	68	144
DDT 100	2.52	.32	2.26	44	65
1,000	2.45	.27	2.21	84	186
2,500	2.57	.26	2.21	54	140
Dieldrin 50	2.63	.27	2.21	67	174
500	2.56	.27	2.13	36	93
L.S.D. at 5% level	.28	.10	.31	41	83

	r Values	
	DDT Levels	Dieldrin Levels
$r_{.05}$	.576	.666
%N with *P. ulmi*	−.244	−.028
%P with *P. ulmi*	+.121	+.522
%K with *P. ulmi*	+.152	−.320
%N with *T. telarius*	−.047	−.290
%P with *T. telarius*	−.107	−.192
%K with *T. telarius*	+.183	+.152

significantly by dieldrin. In 1957 DDT at 100 pounds per acre effected an increase over the untreated check which, however, was not significant (table 1). Total dry weight of defoliated trees, determined in 1957 only, was not significantly affected although a slight stimulatory effect was produced by BHC at 20 pounds (618 grams mean weight per replicate) over the untreated check (588 grams).

The changes in chemical composition of the apple tree were less striking than those brought about by soil insecticides on beans, soybeans, and cotton which may be expected because the apple tree is a slower growing, woody plant. Population development was affected by the insecticides, usually in ways which could be explained in terms of NPK absorption.

In 1956 DDT on fertilized soil did not significantly change the NPK composition of the apple foliage itself (table 2). The changes that occurred, however, were sufficient to effect population differences of considerable magnitude. DDT at 100 pounds per acre depressed populations of both species (fig. 1) and at 1,000 pounds raised the populations of both above the check and significantly above the 100-pound level. It is notable that on the 100-pound level apple foliage contained 0.32% phosphorus, which is above the range of phosphorus previously shown to be favorable to *T. telarius* in apple foliage (Rodriguez 1958). Considering all levels of DDT addition, a negative relationship was apparent between both mite species and per cent phosphorus that year (fig. 1). In 1957 DDT at both 100 and 4,000 pounds per acre on unfertilized soil significantly increased per cent nitrogen over the untreated check and also increased the populations (table 3). The relationship of both mite species with all the absorbed elements was more or less positive (fig. 2). The most striking finding was a significant positive correlation coefficient between per cent phosphorus and *T. telarius* at a range of phosphorus generally below 0.20%. This result further confirms the previous postulation that *T. telarius* populations respond positively to increases in absorbed phosphorus up to 0.20% and may respond adversely to higher levels. In the case of these apple trees, grown in soil under semifield conditions with the addition of soil insecticides, no evidence of nitrogen-phosphorus antagonism was discovered such as was evident in apple trees grown in the greenhouse in nutrient culture on various nitrogen and phosphorus levels (Rodriguez 1958).

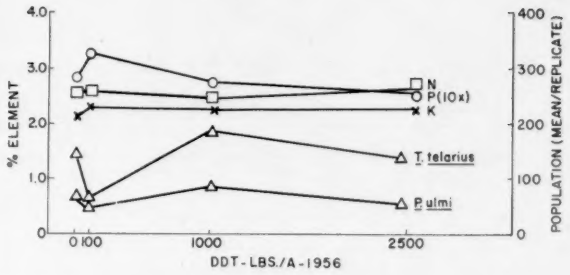

FIG. 1.—Effect of DDT applied to the soil on the NPK absorption in apple trees and on the mite populations developing thereon. Phosphorus is expressed as 10 times the actual amount. Populations are expressed as the average number of progeny developing from five young adult females of *Tetranychus telarius* in 8 days, *Panonychus ulmi* in 10 days. 1956.

Table 3.—Average per cent absorption of elements in foliage of apple trees grown in soil without fertilizer addition and treated with soil insecticides and resulting mite development. 1957.

Insecticide (Lb./A.)	Per Cent Absorbed Element			Average Mites per Leaf Disk	
	N	P	K	*P. ulmi*	*T. telarius*
Untreated	1.94	0.17	1.41	60	180
DDT 100	2.24	.20	1.53	89	203
4,000	2.35	.19	1.52	74	254
BHC 20	1.96	.17	1.56	68	144
200	2.15	.17	1.74	64	141
L.S.D. at 5% level	.28	.05	.39	26	83

	r Values	
	DDT Levels	BHC Levels
$r_{.05}$	.666	.666
%N with *P. ulmi*	+.390	−.025
%P with *P. ulmi*	+.320	+.059
%K with *P. ulmi*	+.577	+.074
%N with *T. telarius*	+.279	+.499
%P with *T. telarius*	+.729*	+.627
%K with *T. telarius*	+.585	−.109

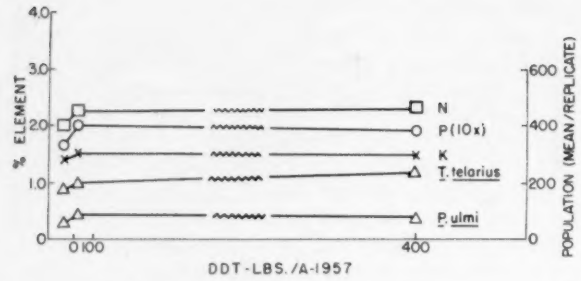

FIG. 2.—Effect of DDT applied to the soil on the NPK absorption in apple trees and on the mite populations developing thereon. Phosphorus is expressed as 10 times the actual amount. Populations are expressed as the average number of progeny developing from five young adult females of *Tetranychus telarius* in 8 days, *Panonychus ulmi* in 10 days. 1957.

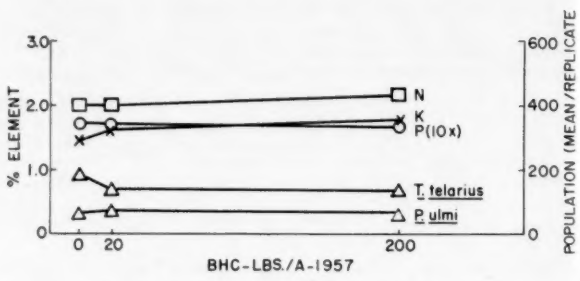

FIG. 4.—Effect of BHC applied to the soil on the NPK absorption in apple trees and on the mite populations developing thereon. Phosphorus is expressed as 10 times the actual amount. Populations are expressed as the average number of progeny developing from five young adult females of *Tetranychus telarius* in 8 days, *Panonychus ulmi* in 10 days. 1957.

Rather, a notable increase in nitrogen generally was concomitant with an increase in phosphorus.

In 1956 dieldrin at 50 pounds per acre increased *T. telarius* over the check and at 500 pounds depressed both species below the check and the 50-pound level (fig. 3). The only correlation coefficient of appreciable value was that of per cent potassium with *P. ulmi* expressing a positive relationship (table 2).

In 1957 BHC exerted virtually no effect on *P. ulmi* populations but depressed *T. telarius* at both the 20- and 200-pound levels (fig. 4). The *T. telarius* population was positively related to per cent nitrogen and phosphorus, appreciably but not significantly (table 3). This effect is not immediately visible in the mean values but appeared in the individual tree reactions.

The evidence presented here on the effects of soil insecticides on the NPK nutrition of apple trees indicates that these plants are affected, as were Black Valentine beans, soybeans, and cotton. The buildup of insecticide residues on the orchard floor should be considered as a potential factor influencing the mite population of orchards. DDT at 100 pounds per acre has been shown to be capable of significantly affecting both the chemical composition of the apple tree and the mite populations developing thereon. In a normal DDT spray schedule on the average commercial orchard the actual amount of DDT applied in one season has totaled almost 100 pounds per acre. It has also been evident in this work that insecticide effects on both composition of foliage and populations may vary with the fertility level of the soil which to some extent determines the fundamental NPK composition of the foliage.

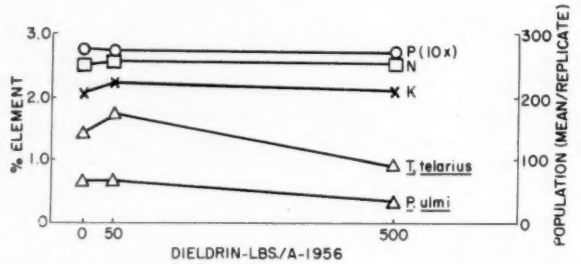

FIG. 3.—Effect of dieldrin applied to the soil on the NPK absorption in apple trees and on the mite populations developing thereon. Phosphorus is expressed as 10 times the actual amount. Populations are expressed as the average number of progeny developing from five young adult females of *Tetranychus telarius* in 8 days, *Panonychus ulmi* in 10 days. 1956.

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Effects of Soil Insecticides and Absorbents on Plant Sugars and Resulting Effect on Mite Nutrition[1]

J. G. Rodriguez, Donald E. Maynard, and Walter T. Smith, Jr.,[2] *University of Kentucky, Lexington*

Abstract

The effects of DDT and various absorbent materials as soil additives on the composition of Black Valentine beans and Clark soybeans and on the *Tetranychus telarius* (L.) populations developing thereon were investigated in the greenhouse. Treatments included DDT only at 800 or 1,600 pounds per acre and DDT at both levels in combination with six levels of the absorbent material as well as an untreated check. Mite development was measured by counting the progeny developing over a period of 8 days from five young adult female mites introduced to a detached leaf disk, each disk being one of four replicates.

Dry weight of foliage was calculated and foliage was analyzed for total and reducing sugar, total nitrogen, phosphorus, and potassium. Analyses for correlation coefficient (r) were made on populations with the results of the foliage analyses.

In Black Valentine beans DDT added to the soil at both 800 and 1,600 pounds per acre increased total and reducing sugars, nitrogen and *T. telarius* populations over the untreated check, while decreasing phosphorus, potassium, and dry weight of foliage. Addition of Hydrodarco B, Aqua Nuchar A, and Nuchar C-115-A generally restored the chemical components of the beans and also the mite populations to the levels of the untreated check. Mite populations were thus correlated positively with reducing sugar and nitrogen, in some cases total sugar, and negatively with per cent phosphorus, potassium, and dry weight of foliage. Peat moss, calcium hydroxide, and Fisher activated charcoal pellets did not function effectively as absorbents; Norit-A and Mallinckrodt activated charcoal alleviated the phytotoxic effects of DDT but did not influence mite populations consistently.

Clark soybeans were not as sensitive to DDT as Black Valentine beans. The percentages of reducing and total sugar were elevated much less and lower concentrations of absorbent were required to reduce these values to the original levels. Hydrodarco B was the only absorbent to affect the chemical composition and mite populations as consistently as in Black Valentine beans although similar trends were noted with Aqua Nuchar A and Nuchar C-115-A.

These studies demonstrated that at least one organic constituent of the plant, reducing sugar, has been markedly influenced in the plant by the addition of high levels of DDT to the soil. Also it has been shown that these plant sugars can be related to the development of a plant feeding mite such as *T. telarius*.

This paper is the last of a series dealing with the general subject of mite nutrition and its relationship to the nutrition of the host plant. In the first paper (Rodriguez *et al.* 1957) the fact was established that the addition of the chlorinated hydrocarbon insecticides to soil affected the nitrogen, phosphorus, and potassium composition of Black Valentine beans, soybeans, and cotton and also the populations of the two-spotted spider mite, *Tetranychus telarius* (L.), and the tumid spider mite, *T. tumidus* Banks, developing on these host plants. The second and third papers gave results of comparative nutrition studies of *T. telarius* and the European red mite, *Panonychus ulmi* (Koch), on apple trees grown in nutrient culture or in soil treated with insecticides (Rodriguez 1958, Rodriguez *et al.* 1960).

The authors have always recognized that the inorganic element composition of the host plant represented only a part of the total picture. Obviously, organic composition needs investigation also. Accordingly, some exploratory measurements of glucose content of expressed sap from Black Valentine beans and soybeans were made, and it was found that there existed differences brought about by soil insecticide treatments (Chen 1957). The purpose of the present study was to investigate the effects of two levels of DDT added to the soil on the growth and chemical composition of Black Valentine beans and Clark soybeans in the presence of various absorbent materials and the resulting effects on populations of *T. telarius*.

Experimental.—*Greenhouse Procedure.*—The host plants, Black Valentine beans and Clark soybeans, were grown in 6-inch pots in screened top soil (Maury silt loam) as described in a previous publication and the treatments were calculated in the same manner. (Rodriguez *et al.* 1957). Preliminary experiments indicated that 800 pounds of DDT per acre consistently caused pronounced changes in plant development; therefore, 800- and 1,600-pound levels of DDT in 50% wettable powder were added to the potted soil. The absorbent materials added to the soil included peat moss, calcium hydroxide, and the following types of activated carbons: Norit-A and Fisher activated charcoal pellets (Fisher Scientific Company), activated charcoal (Mallinckrodt Chemical Works), Nuchar C-115-A and Aqua Nuchar A (West Virginia Pulp and Paper Company, Tyrone, Pennsylvania), and Hydrodarco B (Atlas Powder Company, Wilmington, Delaware). Under our experimental conditions, peat moss, calcium hydroxide, and the first three activated charcoals listed did not appear in the early work to be effective in counteracting the DDT effects on plant sugars or mite populations, and their use was abandoned. (Their effects will be considered briefly in the discussion.) Comprehensive experiments were carried out only with Nuchar C-115-A, Aqua Nuchar A, and Hydrodarco B. The levels of absorbent materials ranged from 1 to 30 tons per acre.

An experiment generally consisted of two replicates (pots of bean plants), each of eight treatments. The treat-

[1] This study was sponsored in part by a grant-in-aid from the Rockefeller Foundation. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director. Partial cost of publication of this paper was met by the Department of Entomology and Botany, Kentucky Agricultural Experiment Station. Accepted for publication March 18, 1960.

[2] Associate Professor of Entomology, former graduate student, and Professor of Chemistry, respectively.

ments included an untreated check, an insecticide-treated check, and six levels of the absorbent material added to the insecticide-treated soil.

Foliage Sampling Procedure.—The primary leaves of the host plant were collected as soon as the trifoliate leaves began to develop, usually 12 to 14 days after planting. The leaves of two-replicates on each treatment in a given experiment were combined, air-dried overnight at 95° C., ground to 40-mesh in a Wiley mill, and stored in tightly covered screw cap jars for chemical analyses.

Methods of Analysis.—Glucose determinations: In preliminary studies fresh foliage samples were treated according to the procedure described by Chen (1957) and glucose content estimated by the use of Tes-Tape (a product of the Eli Lilly Company). In subsequent work a more accurate method of analysis which could be used to determine both reducing sugars and total sugars was adopted. This method, suggested by Griffith & Ellis (1958), was modified and applied to bean foliage analysis as described by Maynard (1958).

Nitrogen, Phosphorus, and Potassium Determinations.—The dried foliage was analyzed for these elements using standard procedures adapted for use in the University of Kentucky Agronomy Department (Todd 1955).

Mite Procedure.—The mites were cultured on leaves detached from the first trifoliate leaf on both Black Valentine beans and soybeans. The original detached leaf culture method (Rodriguez 1953) was modified in that the leaf disks rested on water-saturated filter paper instead of being floated on sucrose solution. Two leaflets (replicates) per pot were detached and since each pot was one of two replicates on each soil treatment, as far as the mite counts were concerned each treatment was actually replicated four times. Five young adult mites were introduced to each leaf disk and cultured in the laboratory at 80° F., about 70% relative humidity and a 15-hour photoperiod. After a developmental period of 8 days the resulting progeny were counted.

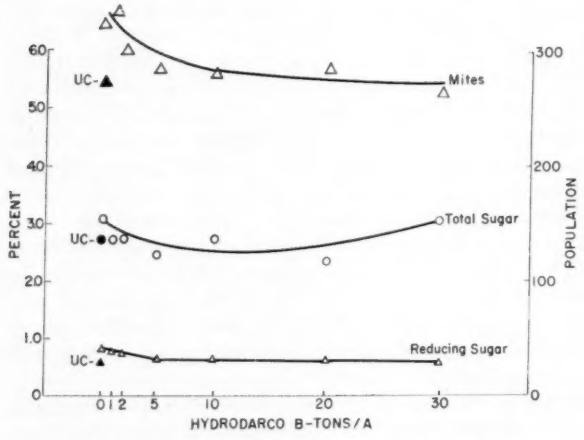

FIG. 1.—Effect of DDT added to the soil and absorbent Hydrodarco B on total sugar, reducing sugar and *Tetranychus telarius* populations on Black Valentine beans. DDT was applied at 800 pounds per acre on all treatments except the untreated check (UC) shown in solid black symbols. Mite population is expressed as the average number of progeny resulting from five females during a developmental period of 8 days.

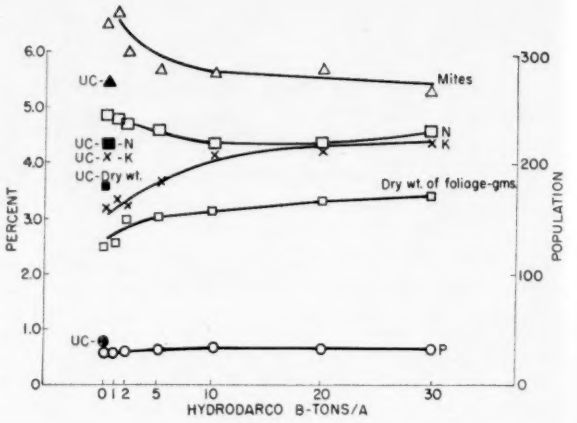

FIG. 2.—Effect of DDT added to the soil and absorbent Hydrodarco B on nitrogen, phosphorus, potassium, dry weight of foliage, and *Tetranychus telarius* populations on Black Valentine beans. DDT was applied at 800 pounds per acre on all treatments except the untreated check (UC) shown in solid black symbols. Mite population is expressed as the average number of progeny resulting from five females during a developmental period of 8 days.

Statistical Analyses.—The mean value of the four replicates on each treatment was calculated for the mite populations. Analysis for correlation coefficient (r) was made with the mean mite population as one variable, and, in turn, each of the values for reducing and total sugars, dry weight of foliage, per cent nitrogen, phosphorus, and potassium obtained from the analyses of combined foliage. Each test generally consisted of eight pairs, one pair on each treatment in each experiment.

RESULTS.—The effects of DDT at 800 pounds per acre on the sugar and NPK composition of Black Valentine beans and on the mite populations are shown in figures 1 and 2 and resemble closely effects obtained when the rate of insecticide was doubled (table 1). The absorbent used in this experiment was Hydrodarco B, which counteracted the DDT effects in a manner typical of the three absorbents tested. DDT added to the soil at 800 or 1,600 pounds per acre increased both total and reducing sugars, nitrogen and *T. telarius* populations over the untreated check, while decreasing phosphorus, potassium, and dry weight of foliage from the untreated check. The addition of carbons generally restored the chemical components of the beans, and accordingly the mite populations, to the level of the untreated check. The restorative effects of Aqua Nuchar A and Nuchar C-115-A appeared at somewhat higher levels than were necessary when Hydrodarco B was used. Table 2 lists the correlation coefficients calculated on the chemical components and dry weight of foliage of the beans with the mite populations. As the curves on the graphs would indicate, the mite populations were correlated positively with reducing sugars and nitrogen and in some cases with total sugars and negatively with per cent phosphorus, potassium, and dry weight of foliage.

Figures 3 and 4 depict the results of the same type experiment on Clark soybeans. Again results between the

Table 1.—Effect of DDT added to the soil and absorbent Hydrodarco B on total and reducing sugar, absorbed elements, dry weight of foliage, and *Tetranychus telarius* populations on Black Valentine beans and Clark soybeans.

Treatment	Absorbent	Tons per Acre	Dry Weight Foliage (Gm.)	Per Cent Sugar[a]		Per Cent			Mites[b]
				R	T	N	P	K	
			Black Valentine beans						
Check: untreated			3.55	0.55	2.70	4.35	0.698	4.30	270
Check: DDT, 50% WP, 800 lb./A.			2.42	0.79	3.05	4.85	0.562	3.10	327
DDT, 50% WP, 800 lb./A.	Hydrodarco B	1	2.49	0.69	2.72	4.75	0.562	3.25	335
	do	2	2.94	0.68	2.71	4.65	0.586	3.19	303
	do	5	2.99	0.63	2.43	4.55	0.582	3.72	287
	do	10	3.11	0.62	2.68	4.35	0.584	4.13	278
	do	20	3.29	0.60	2.33	4.35	0.617	4.28	285
	do	30	3.40	0.53	3.06	4.55	0.617	4.48	267
Check: DDT, 50% WP, 1,600 lb./A.			2.11	1.32	4.47	5.15	0.517	2.94	320
DDT, 50% WP, 1,600 lb./A.	Hydrodarco B	1	2.17	1.30	4.09	5.05	0.628	2.89	305
	do	2	2.19	0.67	2.83	4.95	0.684	3.34	281
	do	5	2.28	0.67	3.04	4.95	0.673	3.34	288
	do	10	2.84	0.63	2.53	4.95	0.684	3.63	287
	do	20	3.12	0.59	2.65	4.85	0.678	4.38	262
	do	30	3.30	0.58	2.29	4.85	0.684	4.32	243
			Clark soybeans						
Check: untreated			2.18	0.63	1.48	5.60	0.544	3.91	189
Check: DDT, 50% WP, 800 lb./A.			1.30	0.76	2.12	6.00	0.582	3.67	257
DDT, 50% WP, 800 lb./A.	Hydrodarco B	1	1.79	0.66	1.66	5.85	0.530	3.84	227
	do	2	1.77	0.64	1.62	5.75	0.540	3.93	231
	do	5	1.78	0.59	1.84	5.75	0.532	3.84	206
	do	10	1.89	0.53	1.48	5.75	0.520	3.88	206
	do	20	2.02	0.56	2.41	5.55	0.500	3.98	194
	do	30	2.07	0.51	1.46	5.45	0.490	3.93	176
Check: DDT, 50% WP, 1,600 lb./A.			1.68	0.89	1.57	6.15	0.540	3.63	206
DDT, 50% WP, 1,600 lb./A.	Hydrodarco B	1	1.77	0.77	1.65	5.90	0.540	3.73	195
	do	2	1.88	0.65	1.32	5.85	0.530	4.04	167
	do	5	1.96	0.60	1.48	5.75	0.490	3.92	162
	do	10	1.96	0.59	1.60	5.55	0.480	4.03	163
	do	20	2.21	0.61	1.55	5.60	0.484	3.93	153
	do	30	2.31	0.55	1.39	5.65	0.490	3.91	169

[a] R, reducing; T, total.
[b] Mean progeny developing from five young adult female mites on one leaf disk during a period of 8 days.

800- and 1,600-pound DDT rates were quite similar in reaction (table 1). The soybean plants were not as sensitive to DDT as the Black Valentine beans. The percentages of reducing and total sugar were elevated much less than in Black Valentine beans and lower concentrations of carbon were required to reduce these values to the original levels. In this host plant nitrogen and phosphorus were absorbed concomitantly rather than antagonistically, phosphorus being increased by 800 pounds DDT and being reduced by carbon additions. This effected a positive correlation of per cent phosphorus with mite populations (table 2). Hydrodarco B was the only carbon to affect the chemical composition of soybeans as consistently as it did Black Valentine beans although trends in the same direction were noted when Aqua Nuchar A and Nuchar C-115-A were used.

DISCUSSION OF RESULTS.—Preliminary work by Chen (1957) with DDT, BHC and lindane, dieldrin, aldrin, and chlordane additions to Black Valentine beans grown in pots in the greenhouse showed glucose generally increasing as the insecticide was increased. Of these materials the plant was the least sensitive to DDT. In the preliminary tests in the present study DDT was added to soil in which Black Valentine beans were planted at 10, 100, 200, 400, 800, and 1,600 pounds per acre; per cent glucose was more than doubled at the 400-pound rate and tripled at the 800-pound rate. These measurements, however, were made by the "Tes-Tape" semiquantitative method of determining glucose (Maynard 1958). When a quantitative method was adopted for reducing and total sugars, the changes were not nearly as great although the percentages of reducing sugars were higher (table 1). While 800 pounds of DDT per acre is an unrealistically high level, it could conceivably be accumulated in 8 to 10 years under conditions such as exist in a commercial orchard because DDT is a very stable insecticide in the soil (Fleming 1950, Polivka 1959).

The absorbents added to the soil at the time of planting the beans had a relatively short time to react in the soil before the leaf disks were detached (about 2 weeks

Table 2.—Values of correlation coefficient (r) comparing percentages reducing and total sugars, elements and dry weight of foliage with mite populations developing on Black Valentine beans and soybeans treated with soil DDT and absorbents.

Plant	DDT (Lb./A.)	Absorbent	Per Cent Sugar[a]		Per Cent Element			Dry Weight of Foliage
			R	T	N	P	K	
B.V. beans	800	Nuchar C-115-A	+.595	+.119	+.808*	−.789*	−.897**	−.547
	1,600	do	+.959**	+.644	+.757*	−.841**	−.994**	−.905**
	800	Aqua Nuchar A	+.825*	+.331	+.828*	−.805*	−.841**	−.822*
	1,600	do	+.685	−.134	+.766*	−.575	−.829*	−.796*
	800	Hydrodarco B	+.888**	+.256	+.871**	−.696	−.922**	−.960**
	1,600	do	+.828*	+.886**	+.569	−.765*	−.907**	−.795*
Soybeans	800	Nuchar C-115-A	+.579	+.320	.004	+.053	.005	+.508
	1,600	do	−.014	−.237	+.334	+.187	−.369	−.179
	800	Aqua Nuchar A	+.355	+.399	+.420	+.340	+.353	−.360
	1,600	do	+.392	−.252	+.507	+.273	−.443	−.307
	800	Hydrodarco B	+.853**	+.273	+.918**	+.793*	−.768*	−.931**
	1,600	do	+.839**	+.238	+.747*	+.894**	−.768*	−.610

[a] R, reducing; T, total.
r .05 = .707
r .01 = .834

in Black Valentine beans and 4 weeks in Clark soybeans). Peat moss and calcium hydroxide did not show any effect on glucose content and tended to depress the foliage weights or plant growth. Addition of activated carbon to the soil alleviated the phytotoxic effects of the DDT and reduced the glucose in all cases except where Fisher activated charcoal was used. However, this charcoal was in pellet form and the other carbons were in powder form. The only types of absorbents having consistent effects on mite populations were Aqua Nuchar A, Nuchar C-115-A, and Hydrodarco B. The first two are acid washed, activated carbons whereas the latter is an alkaline, unwashed carbon. Aqua Nuchar A and Hydrodarco B are recommended for use in municipal water supplies and sewage systems. Nuchar C-115-A is twice activated and more refined than the other two. It appears that the most effective absorbent for our purposes was not necessarily the most expensive or most refined.

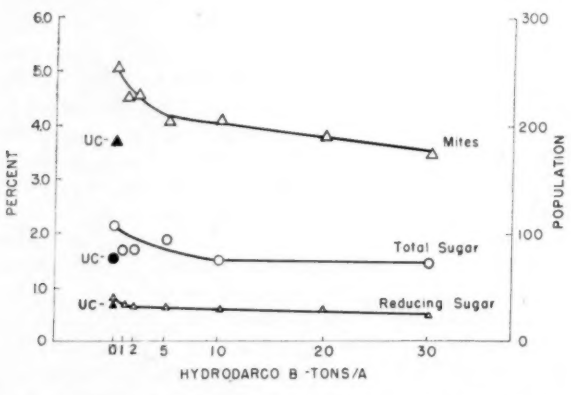

FIG. 3.—Effect of DDT added to the soil and absorbent Hydrodarco B on total sugar, reducing sugar, and *Tetranychus telarius* populations on Clark soybeans. DDT was applied at 800 pounds per acre on all treatments except the untreated check (UC) shown in solid black symbols. Mite population is expressed as the average number of progeny resulting from five females during a developmental period of 8 days.

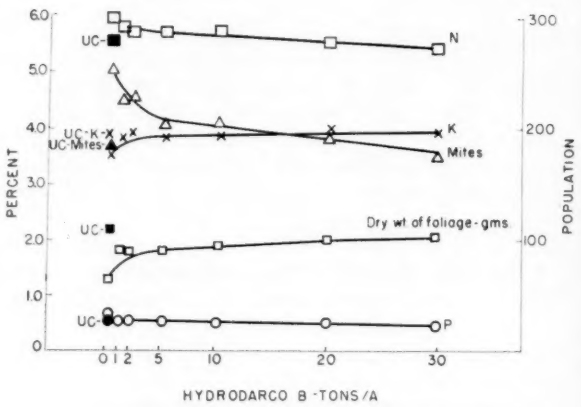

FIG. 4.—Effect of DDT added to the soil and absorbent Hydrodarco B on nitrogen, phosphorus, potassium, dry weight of foliage, and *Tetranychus telarius* populations on Clark soybeans. DDT was applied at 800 pounds per acre on all treatments except the untreated check (UC) shown in solid black symbols. Mite population is expressed as the average number of progeny resulting from five females during a developmental period of 8 days.

In the experiments described here the reactions of *T. telarius* populations were generally more uniform and less variable than in some earlier work. In the past interrelations among ions and differences in level of NPK absorption in the same host plant under different conditions have produced relationships between elements and populations which could not always be explained solely on the basis of inorganic nutrition. These studies were an effort to explore the organic nutrition of the host plant and its relation to mite development. Our results demonstrate conclusively that at least one organic constituent, reducing sugar, is influenced when the plant is grown in soil having

a high level of DDT and that this type of organic nutrition is also involved with the development of a plant-feeding mite such as *T. telarius*.

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Residues of Malathion on Alfalfa and in Milk and Meat[1]

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ABSTRACT

Residues of malathion, applied at 1 pound actual per acre to alfalfa 11.4″ tall, decreased from an average of 69 p.p.m. immediately after application to 0.25 p.p.m. 6 days later. Milch cows fed malathion at 8 p.p.m. for 3 weeks did not excrete the insecticide in milk nor was it detectable in the blood, brain, liver, kidney, round or rib-eye when the animals were slaughtered at the conclusion of the experiment.

The fate of malathion, *S*-[1,2-bis(ethoxycarbonyl) ethyl] *O,O*-dimethyl phosphorodithioate, ingested by animals or dermally applied, has received some attention. March *et al.* (1956a, 1956b) have studied P^{32}-labelled malathion, sprayed on hens and heifer-calves, while Cook (1957) and subsequently Ahmed *et al.* (1958) have investigated the *in vitro* effect of bovine rumen fluids on various organophosphate pesticides. Gjullin *et al.* (1955) grazed cows on alfalfa pastures sprayed with known amounts of malathion and subsequently failed to detect any in the milk. However, in this instance it is unknown how much of the malathion was actually consumed by the cows.

The paucity of available information thus made it desirable to undertake a further study of this problem in 1957, as a contribution to Federal North East Project No. 36 (NE 36) "Pesticide Residues on Agricultural Crops." It was decided to determine the rate of disappearance of malathion under local conditions after spraying alfalfa, and to find out if residues could be detected in the milk and tissues of cows fed at Food and Drug Administration (FDA) tolerance levels for some time.

RATE OF DISAPPEARANCE OF MALATHION ON ALFALFA.—On August 22 alfalfa plots 1 and 2 were sprayed with malathion from an emulsifiable concentrate at the rate of 1 lb. per acre, with plot 3 used as an unsprayed control. The height of the plants averaged 11.4″.

Since Smith *et al.* (1954, 1955) have stated that the stability of malathion on plants can be affected by temperature, wind, and rain, it should be noted that weather conditions at the time of application were: temperature 68° F., relative humidity 80%, partly cloudy. During the experiment the mean daily temperature varied from 60° to 70°, with a maximum of 80° and a minimum of 43°, and rainfall amounting to 0.24″ fell on August 25, 1957.

On the days of harvest, the alfalfa was cut, brought to the laboratory and solvent-stripped immediately, with prompt subsequent analysis by the method recommended by H. W. Conroy (Federal Food & Drug Administration 1957). The results given in table 1 are on the green alfalfa as cut.

MALATHION RESIDUES IN MILK.—In this experiment two Holsteins, cow A weighing 1230 pounds and cow B weighing 1100 pounds, were fed known amounts of malathion, while a third cow C was used as a control. The insecticide was sprayed directly on the alfalfa being fed at milking time, about 4:00 p.m., and careful watch was made that all was consumed. The morning feeding was unsprayed. The amount of malathion fed was calculated to approximate the maximum tolerance set by Food and Drug Administration for this material on alfalfa as consumed in normal-forage intake, namely 8 p.p.m. The malathion was derived from a 57% emulsifiable concentrate, mixed with sufficient water to give coverage to the alfalfa, care being taken that all was sprayed on the alfalfa.

Cow A received 0.14 ml. of 57% malathion (5 lb./gal.) emulsifiable concentrate each day from July 15 to August

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Table 1.—Rate of disappearance of malathion sprayed August 22 on alfalfa. Amherst, Massachusettes. 1957.

Date of Sampling (Aug.)	Residue of Malathion (p.p.m.)	
	Plot 1	Plot 2
22	72	66
24	25	26
26	1.2	1.1
28	.25	.27

10 inclusive, for a total cumulative intake of 3.78 ml. (2.26 gm. malathion).

Cow B received 0.13 ml. of 57% malathion (5 lb./gal.) emulsifiable concentrate each day from July 15 to August 4 inclusive, for a total cumulative intake of 2.75 ml. (1.65 gm. malathion).

Cow C received no malathion.

During the period of test, samples from the morning and evening milking were taken, generally every other day, and 1-liter samples from each were treated in a freeze-drying apparatus under vacuum. A total of 16 of these 1-liter samples from the two malathion-fed cows, dispersed throughout the test period, were extracted and analyzed by the method of Conroy (1957). In no case was any malathion detectable.

Prior to the start of the experiment, a 1-liter sample of herd milk was taken at random, and to this was added 0.258 mg. of malathion. Recovery of malathion by the procedure cited was 0.20 mg., or about 78%.

On August 13, a sample of milk was again taken from cow A. One liter of this milking was used as a control, analyzed, and no malathion found. To a second liter of this milking was added 0.258 mg. of malathion, and by analysis it yielded 0.22 mg., or a recovery of 85%.

Throughout this feeding experiment, the cows were carefully examined and no unusual symptoms were noted at any time.

Malathion Residues in Meat.—Malathion-fed cow B from the milking experiment above, and control cow C were sacrificed on August 5. The following tissues from cow B were analyzed for malathion: brain, liver, kidney rib-eye, round, blood. In each case, except blood, fat was removed by cutting as completely as possible, 200 gm. of the lean tissue were passed through a meat grinder and extracted with carbon tetrachloride in a Waring Blendor. Blood was extracted directly. The extract was then analyzed by the procedure of Conroy (1957). Malathion was undetectable in any of the six tissues. Because no insecticide was found in the malathion-fed cow, it appeared unnecessary to analyze the tissues from the control cow. At the time of slaughter, a post-mortem examination of cow B was made by a member of the Veterinary Science Department, and no abnormal lesions were noted.

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Dosimetry for Studies on the Radiobiology of *Tribolium castaneum* Using the Van De Graaff Electron Accelerator[1]

John D. Hilchey and Raymond D. Cooper[2,3]

Abstract

A means is given for calculating the dose of radiation, in rads, absorbed by adult *Tribolium castaneum* (Hbst.) when irradiated with high-energy electrons from a Van de Graaff accelerator. The expression for dose is based on (1) the power output of the accelerator; (2) the amount of time the target (insect) is in the beam; (3) the fraction of the scanned area occupied by the target; (4) the fraction of the energy of the incident electrons which is lost in the target; and (5) the mass of the target. The expression is developed in detail on the basis of the ideal case, but possible departures from the ideal in practice are discussed. The physical parameters of the insect which pertain to the calculation of dose are reported.

In carrying out investigations on the feeding activity of irradiated *Tribolium castaneum* (Hbst.) (Tenebrionidae: Coleoptera) it was necessary to determine the amount of ionizing radiation absorbed by the insects when exposed to the beam of a Van de Graaff electron accelerator. Gen-

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eral methods have been given for calculating the doses administered by this instrument (Trump & Van de Graaff 1948, Baker *et al.* 1954a, 1954b). While none of these methods fitted, precisely, the needs of this study, it was possible to use them to develop an expression for absorbed dose that applied directly to the present problem. It should be made clear that this approach does not represent an advance in dosimetry, but it is simply the application of widely known principles to a specific problem. Therefore, while these doses were calculated for whole-body exposures, modifications may be made easily for dosimetry in partial-body exposures.

The major reason for describing these methods in detail is to make known the methods applied by the authors in a form capable of critical examination by others in the biological field. Secondarily, the authors hope to provide useful background information to those entomologists entering the field of radiation biology.

The Van de Graaff electron accelerator, or electrostatic generator as it is sometimes called, is a source of high-speed electrons. By accelerating free electrons through potential differences measured in millions of volts, these electrons are impinged upon a target with a velocity approaching that of light and, consequently, with great kinetic energy. It emits these electrons as a beam of particles having energies that range very narrowly about a known mean value. A stationary target (the item to be irradiated) can be placed in the path of the beam and the beam turned on, or the target can be placed on a conveyor belt and moved under the beam. If the target is too wide for the stationary beam, the beam can be scanned, or moved back and forth across the width of the conveyor belt. When the wide target is run under a beam scanned in this way, the whole target is exposed to the radiation. The scanned beam and conveyor system were used in these studies.

METHODS OF MEASURING RADIATION.—There are many ways of measuring the dose of radiation applied to a target; physical methods include the use of Faraday cages and ionization chambers and the detection of temperature changes; chemical methods include the detection of changes in the composition of carefully standardized chemical systems; bioassay techniques can be used occasionally as secondary standards in cases where the biological effects of radiation are clearly defined.

The doses used in this study were calculated directly on the basis of the physical and biological parameters which affect the amount of radiation absorbed by the insect. The biological assumptions made are identical to those that would be made in devising a physical or chemical unit which would duplicate the qualities responsible for the radiosentitivity of a biological system. By calculating the doses, it is possible to avoid the difficulties inherent in fabricating an appropriate inanimate test instrument.

The doses used are expressed in rads; the rad is the standard unit of absorbed dose and is equal to 100 ergs of energy absorbed per gram of the target (Hine & Brownell 1956). This unit is independent of the nature of the target material. The dose in rads is affected by:

(1) the power output of the machine
(2) the amount of time the target is in the beam
(3) the fraction of the scanned area occupied by the target
(4) the fraction of the incident energy which is transferred to the target
(5) the mass of the target.

EQUATIONS FOR DEVELOPING EXPRESSION OF RADIATION DOSES.—It is possible to develop an expression for absorbed dose in the terms of the parameters listed above. The beam current and beam potential can easily be determined for any setting of the machine. Thus,

$$\text{Power output} = IV \text{ watts} \tag{1}$$

where I is the current in microamperes and V is the potential in millions of volts. Since one watt equals 10^7 ergs per second, then

$$\text{Power} = 10^7 IV \text{ ergs/sec.} \tag{2}$$

The total work put out by the machine while the target is in the beam is equal to the power multiplied by the time the target takes to move across the area scanned by the beam. The time the target is in the beam can be expressed, in seconds, as the width of the scanned area divided by the conveyor speed, as is shown in expression (3):

$$\text{Time} = \frac{w \text{ cm.}}{s \text{ cm./sec.}} \quad \text{or} \quad \frac{w}{s} \text{ sec.} \tag{3}$$

where w is the width of the beam, in centimeters, in the direction perpendicular to the plane of scan, and s is the velocity of the target in centimeters per second. Thus the total energy put out by the machine while the target is crossing the beam is:

$$\frac{10^7 IV \text{ ergs}}{\text{sec.}} \left(\frac{w}{s} \text{ sec.}\right) \quad \text{or} \quad \frac{10^7 IVw}{s} \text{ ergs} \tag{4}$$

In passing through the air between the scanning tube window and the target, the electrons lose energy as a result of collisions with atoms of the atmospheric gases. The distance between the scanner window and the target depends on the needs of the experiment involved, but it should not exceed 20 to 30 cm. in order to keep energy losses to a minimum.

The correction factor for such losses in beam energy can be calculated very accurately, if necessary. However, an approximation useful for the scanner-to-target distances normally employed can be derived from the values given by Hine & Brownell (1956) (table VIII, p. 100). If we consider that: (1) V equals the initial energy of the electrons in Mev, (2) d equals the distance, in centimeters, from the scanning tube window to the target, and (3) $(dT/dx)V$ equals the rate of energy loss in air (in Mev per cm.) by electrons of energy V, due to ionization and radiation; then the factor, v, which corrects for energy loss in air is given by the expression:

$$v = \frac{V - d\left(\frac{dT}{dx}\right)V}{V} \tag{5}$$

For instance, the correction factor for 2 Mev electrons traveling through 20 cm. of air is approximately 0. 98, representing a loss of about 2.2% of the original energy.

Substituting in (4), we find that the beam energy, after losses in air, is:

$$\frac{v10^7 IVw}{s} \text{ ergs} \tag{6}$$

The total energy after losses is absorbed in the area which is scanned by the beam at a rate of 200 cycles per second. Although the beam is well defined within the machine, many of the electrons are scattered by the exit window and the air; thus the beam does not deliver its electrons in a uniformly dense pattern on the conveyor. Despite this fact the dose is integrated for a portion of the conveyor width simply because of the overlapping effect caused by scanning. There is a sharp decrease in the electron density at both ends of the scanning cycle. It is possible to determine the portion of the total energy which falls along the straight-line region of the curve of electron density in the plane of scan by determining the ratio, E, of the partial integral of the straight-line region of the curve to the total integral under the curve. The incorporation of this factor yields an expression for usable energy output of the beam:

$$\frac{Ev10^7IVw}{s} \text{ ergs} \qquad (7)$$

In cases where the width of the target must be greater than the length of straight-line region of the curve of electron density, the ratio of the appropriate partial integral to the total integral of the curve must be calculated in order to determine the usable portion of the total energy output. The appropriate partial integral would be defined by the width of the particular insect container used. In this case, the true dose will vary systematically across the width of the container; however, the calculations will yield only the integrated, or average, dose across the container. For this reason it is advisable, when possible, to so design the insect container that its width is no greater than the length of the straight-line region of electron density in the plane of scan. The distribution of electrons on the conveyor belt also varies in the direction in which the conveyor belt, or the target, moves. However, since the target moves through this region of variable electron density, the dose applied to the target is integrated, that is, the target may be said to receive some average dose while it is in the beam.

This effect holds true for conveyor speeds normally used for such tests, *i.e.*, up to 10 cm. per second. At these belt speeds the cyclic rate of scan, 200 cycles per second, provides 40 passes of the beam for each centimeter of travel by the target. This gives an overlap of successive passes of the beam that is sufficient to provide the integrating effect.

As previously noted, scattering effects cause the beam to scan an area rather than a line. The electrons put out by the machine are distributed over that area. Since the dose administered to the target is integrated by movement of the target through the scanned area, the fraction of the usable electrons which hits the target may be expressed by the ratio of the target area to the usable portion of the scanned area. If the energy of the electrons is 2 Mev or more, the scanned area tends to be rectangular under the straight-line region of the curve of electron density for the plane of scan. Thus, for very narrow insect containers the ratio of target area to usable scanned area would be:

$$\frac{a \text{ cm.}^2}{lw \text{ cm.}^2} \quad \text{or} \quad \frac{a}{lw} \qquad (8)$$

where a is the area of the target insect in square centimeters, l is the length in centimeters of the region of uniform electron density in the plane of scan, and w is the width of the scanned area in centimeters. Substituting in the general expression (7), the maximum energy which could be transferred by the electrons hitting the target is given by equation (9):

$$\frac{Ev10^7IVwa}{slw} \quad \text{or} \quad \frac{Ev10^7IVa}{sl} \text{ ergs} \qquad (9)$$

If the energy of the electrons is 1 Mev or less, the scanned area tends to be elliptical because of the scattering. In this case three possible situations exist:

(a) If the width of the insect container is no wider than the length of the region of uniform electron density in the plane of scan, the usable scanned area will be like a truncated ellipse. The form of this figure probably approaches that of a rectangle so closely that equations (8) and (9) can be used;

(b) If the width of the insect container is so large that practically the whole width of the scanned area is occupied by the container, the usable scanned area should be considered an ellipse and the relationship of target insect area to usable scanned area is as given in expression (8A):

$$\frac{4a \text{ cm.}^2}{\pi lw \text{ cm.}^2} \quad \text{or} \quad \frac{4a}{\pi lw} \qquad (8A)$$

where a is the area of the target insect and l and w are, respectively, the major and minor axes of the ellipse. Substituting in equation (7) we get:

$$\frac{Ev10^7IVw4a}{s\pi lw} \quad \text{or} \quad \frac{4Ev10^7IVa}{\pi sl} \text{ ergs} \qquad (9A)$$

(c) If the insect container is intermediate in width between the two extremes described in (a) and (b), the area in which the usable electrons fall may have a complex shape which approximates neither an ellipse nor a rectangle. In this situation graphical or mechanical solutions may be used to determine the appropriate scan area to be used in (8) and (9).

If the target is thinner than the maximum range of the electrons used, then only a portion of the electrons which hit the target give up all their energy and come to rest in the target. The remainder pass through. *Tribolium castaneum* adults are thinner than the maximum range of the electrons used. Since only a portion of the electrons that hit the insect give up all their energy to the insect, only a part of the total energy is absorbed by the insect. In order to determine that portion of the available energy that the insect actually receives, three assumptions have been made:

(1) The insects are presented to the beam in an uncovered container, and in a layer which is one beetle thick.

(2) All energy absorbed by the insect causes ionization in the tissues;

(3) The average density of the beetle (table 1) is so close to that of water that the curve of ionization in depth of water can be used as the basis for calculations. The relationship of ionization to depth of water can be shown to follow the curve given in figure 1 (modified from Trump & Van de Graafl, 1948).

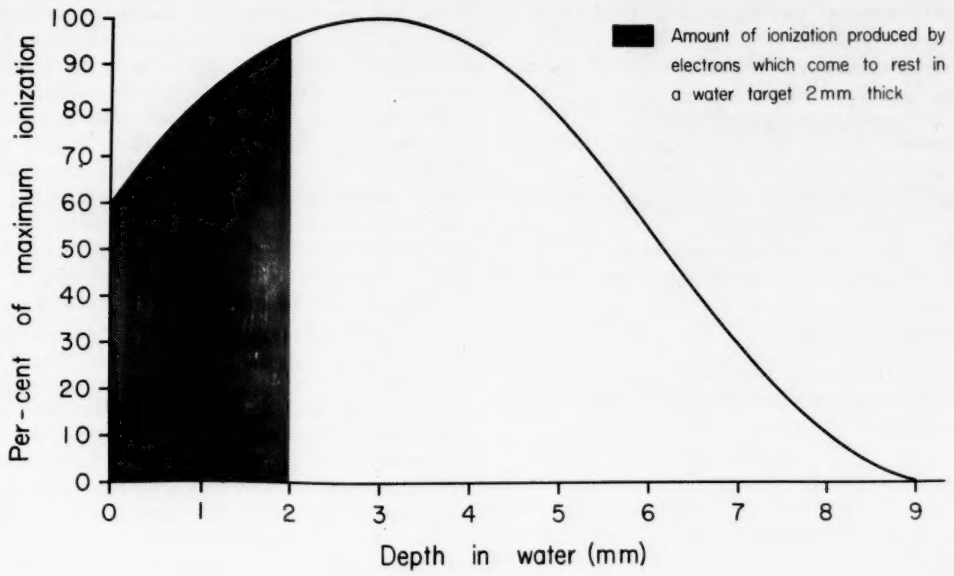

FIG. 1.—Relationship of per cent maximum ionization to depth of water irradiated with 2 Mev. electrons. (Modified after Trump & Van de Graaff 1948.)

The total area under the curve represents the total ionization, or energy transfer, if all electrons are stopped by the target. For a water target thinner than the maximum range of 2 Mev electrons, for instance, a layer of water 2 mm. in depth, the area under the appropriate portion of the curve (shaded area in figure 1) represents the amount of ionization produced by the electrons which hit the target. Therefore, the ratio of the appropriate partial area to the total area under the curve represents the fraction of the total energy which is actually absorbed by the target. If this fraction is represented by f, then the total energy absorbed by the target is expressed by equation (10):

$$\frac{Ev10^7IVaf}{sl} \text{ ergs} \qquad (10)$$

The doses received by the various beetles irradiated in a layer of insects that is more than one individual thick may be calculated by using the partial integrals of the segments of the curve which correspond to the beetles at different depths in this layer. If container covers must be used to restrict the movement of the insects, the losses of energy in the cover can be accounted for by employing curves of ionization in depth which are so derived as to be independent of the nature or density of the material.

Table 1.—Experimentally determined values for biological parameters considered in high-speed electron dosimetry for *Tribolium castaneum*.

Average weight of a beetle	$=0.00182$ gm. ± 0.0000153 gm.[a]
Average volume of a beetle	$=0.00172$ cm.$^3 \pm 0.0000252$ cm.^{3} [a]
Average density of a beetle	$=1.05$ gm./cm.^{3}
Average area of median frontal section	$=0.0336$ cm.$^2 \pm 0.000186$ cm.^{2} [b]
Average effective thickness	$=0.0512$ cm.

[a] Mean and standard error of the mean based on three values; each value equals:

$$\frac{\text{weight (or volume) of 100-beetle sample}}{\text{number of beetles}}.$$

[b] Mean and standard error of the mean based on 300 values; each value is the area of a single beetle.

Part of the electrons of the beam hit the steel conveyor belt rather than the target. A certain small portion of such electrons is backscattered or, in a sense, reflected into the target and, in addition, a certain amount of X-radiation is produced. The makers of the machines[4] estimate that the X-ray production is less than 1% of the dose received directly from the beam. Therefore, this factor is ignored in the expression for dose. The effect of back-scatter is also considered to be too minor a factor for inclusion in the computations.

The unit of absorbed dose, the rad, is expressed in units of energy per unit of mass. Therefore, if the target weighs g grams, then the energy absorbed in ergs per unit mass of the target is shown in expression (11):

$$\frac{Ev10^7IVaf}{sl}\left(\frac{1}{g}\right) \text{ ergs/gm. or } \frac{Ev10^7IVaf}{slg} \text{ ergs/gm.} \qquad (11)$$

Since 1 rad equals 100 ergs per gram, then the absorbed dose for a whole-body exposure in a beam characterized by an essentially rectangular area of usable scan is given by equation (12):

$$\frac{Ev10^5IVaf}{slg} \text{ rads or } I\left(\frac{Ev10^5Vaf}{slg}\right) \text{rads} \qquad (12)$$

where:

I = beam current in microamperes (μa).
E = factor representing the fraction of the total energy falling within the usable scanned area.
v = factor to correct for energy loss by electrons in air.

[4] High Voltage Engineering Corp., Burlington, Massachusetts.

10^5 = factor to account for the product of two arithmetical conversions:
(1) watt seconds to ergs (10^7)
(2) ergs per gram to rads (10^{-2})
V = beam potential in millions of volts.
a = area of the median frontal section of the insect ($cm.^2$).
f = proportion of the energy of the incoming electrons which is lost in the target.
s = speed of the conveyor belt (cm./sec.).
l = length of the usable scanned area in the plane of scan (cm.).
g = weight of the insect (gm.).

Expression (12A) gives the final form of the calculation for absorbed dose in cases where the usable scanned area is considered to be an ellipse:

$$I\left(\frac{4\times10^5 vEVaf}{\pi slg}\right) \text{ or } I\left(\frac{1.27\times10^5 vEVaf}{slg}\right) \text{ rads} \quad (12A)$$

where l is now to be considered the length, in centimeters, of the major axis of the ellipse, and $4/\pi$ or 1.27 is the constant in the expression for the area of an ellipse. If the usable scanned area is other than one of those defined for expression (12) or (12A), appropriate dimensional changes can be made in one of these expressions to account for the deviation.

In preparation for calculating the specific doses administered to *Tribolium castaneum* for these studies, values for beam current, beam potential, conveyor speed, and width and length of scan were determined by the operator at the irradiation facility.

BIOLOGICAL MEASUREMENTS.—The biological constants for *T. castaneum* adults were obtained from three samples of 100 beetles each, selected randomly from a culture, 2 weeks after eclosion. The average live weight of a beetle was determined after direct weighing of each group of 100 insects. The average volume of a beetle was determined after measuring the volume of acetone displaced by each of the above groups of 100 beetles. All 300 beetles were then placed in normal crawling position on frosted glass and photographed by transmitted light in order to prepare photographic enlargements of their silhouettes. The areas of the 300 enlarged silhouettes were determined by a planimeter and the known enlargement factor was used to calculate the average area of a median frontal section of a beetle.

Since a beetle varies in thickness in both the transverse and the longitudinal dimensions, an expression for "average" thickness of the beetle was coined. The term "effective thickness" will be used in referring to this measurement. For the purposes of calculating whole-body doses, the beetle was considered uniformly thick throughout the body. The value for the effective thickness is most easily derived indirectly from the relationship of body volume and the area of the median frontal section. Dividing the average area of the median frontal section into the average body volume of a group of beetles yields the average effective thickness of the body.

The results of the biological measurements and calculations are given in table 1. The proportion of the electrons' total energy which is given up to the target was calculated from the curve of ionization in water (Trump & Van de Graaff 1948). The total area under the appropriate curve and the partial area under that portion of the curve which represented a depth of water equal to the average effective thickness of the beetles were obtained by use of a planimeter. The ratio of the partial area to the total area was used in the dose calculations to express the fraction of the energy of the incoming electrons which is transferred to the target (f). Substitution of the biological and physical data described above in equation (12) gave numerical values for the doses in rads.

An effort has been made to include herein all factors of major importance of which the authors are now cognizant. As new biological factors affecting dosimetric calculations are found, the current scheme for calculation will be revised accordingly. Adequate description of the assumptions and techniques used for dosimetric calculations is an essential requirement if comparisons are to be drawn between the results from different laboratories.

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NOTICE TO AUTHORS

Authors are urged to consult the latest revised list of "Common Names of Insecticides." See JOURNAL OF ECONOMIC ENTOMOLOGY 52(2): 361–362, April 1959 and 52(5): 1032, October, 1959. Reprints are available free upon request to the Office of the Executive Secretary.

The chemical definition of each insecticide, the name of which does not appear in these lists, should be given the first time the name occurs in the abstract and the first time the name occurs in the text of a manuscript accepted for publication by the Entomological Society of America.

Insecticidal Control of the Hessian Fly[1]

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ABSTRACT

Several organophosphate systemic insecticides were field-tested further in 1957, 1958, and 1959 as a control for the Hessian fly, *Phytophaga destructor* (Say). The materials used were: Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate), Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and phorate (formerly known as Thimet). These materials were applied with fertilizer from a standard grain-drill at planting time about 1 month before the fly-free date. Seed treatments were also included in some experiments for comparison. One-pound rates of phorate gave excellent protection from Hessian fly attack over long exposure periods. Materials applied with fertilizer significantly increased both forage and grain yields at the 1-pound rate of application.

In 1957 it was reported that certain systemic organic phosphate insecticides would control the Hessian fly, *Phytophaga destructor* (Say) (Brown 1957). Since then several workers have published on various phases of the problem (Guyer *et al.* 1958, Stanley 1958, Bigger 1959). This report presents data gathered in Missouri during 1957, 1958, and 1959. Three field experiments on control were carried out, one at Pierce City in southwestern Missouri and two at Columbia in central Missouri.

MATERIALS AND METHODS.—The Pierce City experiment was designed to test phorate (formerly known as Thimet) in two formulations and at four dosage levels, ½, 1, 2, and 4 pounds actual toxicant per acre. Both formulations were applied with fertilizer, one as a 90% emulsifiable concentrate adsorbed directly on the fertilizer, the other a 44% powder on carbon as a fertilizer mix. The fertilizer was a standard 12-12-12 applied at the rate of 200 pounds per acre.

The wheat was planted September 17, 1957, approximately 1 month before the fly-free date for the area. The treatments were assigned at random to plots eight drill rows wide by 50 feet long in six replications. An eight-row fertilizer grain drill was used to plant all the experiments. The planting rate was 1 bushel per acre. Infestation was determined from samples of 100 plants taken in December.

The 1957 experiment at Columbia consisted of treatments randomized on plots eight drill rows wide by 50 feet long in five replications. Two varieties of wheat, one susceptible to Hessian fly (Tenmarq) and one resistant (Ponca), were planted on two dates, one on September 7, a month before the fly-free date, and one as close to the fly-free date as weather would permit, October 10. The fly-free date at Columbia is October 6. In this experiment three organophosphate system insecticides, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) (50% on carbon), phorate (90% emulsifiable concentrate), and Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate) (47.5% emulsifiable concentrate) were tested at rates of 1 and 2 pounds ac-actual toxicant per acre. The insecticides were applied with fertilizer as in the previous experiment. A one-half-pound-per-acre phorate seed treatment (44% on carbon plus dexdrin-sorbitol sticker) was also included.

Table 1.—Hessian fly control with two formulations of phorate at four dosage levels. Pierce City, Missouri, 1957.[a]

TREATMENT		AVERAGE NO. OF MAGGOTS 100 PLANTS[b]
Formulation	Pounds of Toxicant/Acre	
Untreated	0	145.50
90% Emulsifiable Concentrate	0.5	10.20
	1	0
	2	0
	4	0
44D Activated Charcoal Powder	0.5	8.50
	1	0.66
	2	0
	4	0

[a] All treatments applied as fertilizer-insecticide mixes on Tenmarq wheat.
[b] Six replications.

Forage yields based on square-yard clippings were taken November 20. The forage yields were taken independently of the grain yields, *i.e.*, all grain yields were harvested from unclipped plants.

Plant samples from the phorate-treated plots were taken October 19 and November 20 and sent to the American Cyanamid Company for residue analysis. Samples of 50 plants per plot were collected in December for determining infestations by the Hessian fly.

Grain yields were obtained during the first week of July 1958. Samples consisted of two 20-foot-long rows from each plot.

The third experiment included rates of 1 pound actual toxicant per acre of Di-Syston (50% on carbon), phorate (44% on carbon) and phorate 10% granules, all blended with 12-12-12 fertilizer. Phorate seed treatment at one-half pound per acre with captan as a safener was included. The treatments were applied at random in four replications. The wheat was planted September 13, 1958.

RESULTS AND DISCUSSION.—The results of the Pierce City experiment are shown in table 1. The wheat was exposed to a moderately heavy infestation of Hessian flies, placing the treatments under sufficient infestation pressure to bring out sharp differences. All rates of both formulations gave good control with even the one-half pound rate, reducing the number of maggots much below that in the untreated checks. There was no significant difference between the two formulations with respect to control. The 2- and 4-pound rates caused considerable damage to the stand; at the higher rate, an estimated reduction of 50% was observed.

Results of the 1957–58 experiment at Columbia are presented in table 2. Only phorate gave complete protection at the 1-pound rate, but all treatments greatly reduced the maggot population. While the grain yields

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Table 2.—Hessian fly control with systemic insecticides on two varieties of wheat and on two dates of planting.[a] Columbia, Missouri, 1957–58.

Treatment	Pounds of Toxicant/A.	Wheat Variety	Maggots/100 Plants	Yields	
				Forage (Lb./A.)	Grain (Bu./A.)
		Early Plantings (Sept. 7)			
Control	0	Tenmarq	54.0	16,215	38.04
Di-Syston	1	Tenmarq	5.0	20,245	45.80
	2	Tenmarq	0.0	22,381	39.70
Phorate 90% E.C.	1	Tenmarq	0.0	23,306	45.92
	2	Tenmarq	0.0	21,605	44.66
Am. Cyanamid 12008	1	Tenmarq	8.3	23,013	43.00
	2	Tenmarq	0.0	19,663	41.35
Seed Treatment phorate 44D	0.5	Tenmarq	13.0	17,478	39.98
Control	0	Ponca	0.5	22,964	38.59
Phorate 44D	2	Ponca	0.0	26,508	46.16
		Fly-Free Date Plantings (Oct. 10)			
No treatment	—	Tenmarq	0.0	—	31.15
No treatment	—	Ponca	0.0	—	44.11
L.S.D. at 1% level				5,844	10.2
at 5% level				4,374	7.7

[a] Five replications.

from plots treated with the 1-pound rates of Di-Syston and phorate were the only ones differing significantly from the untreated checks, all other materials, at all rates of application, increased yields to some extent.

Stimulation of plant growth, which has been noted in previous work (Brown 1957, Guyer *et al.* 1958), was obvious again. Forage yields in the plantings of Tenmarq were increased significantly over the untreated check in four treatments. These differences do not seem to be caused entirely by the control of Hessian flies. The effect may be related in part to the incidental control of associated soil insects. The increase in forage and grain yields of the resistant Ponca when treated with phorate might be explained partially in this way.

The plants in the plots planted with phorate-treated seed were definitely stunted and, although germination was good, growth was conspicuously retarded. The difference between the grain yield in the Tenmarq check plots, planted on the different dates, was not significant at the 5% level.

No phorate residue was found in the 1-pound treatment in samples collected on November 20, 1959, but there was a residue of 0.050 p.p.m. in the samples collected on October 19. The 2-pound rate showed 0.095 p.p.m. on October 19 and 0.037 p.p.m. on November 20.

Data obtained from experiment 3 are shown in table 3. This experiment was carried out under a low infestation of Hessian flies, but under long exposure. An unusually late emergence of flies carried oviposition well into October with larval migration continuing until late October. The plots were infested September 27 and again October 13 with puparia that had been held in cold storage through the summer. The fall was warm with sufficient moisture to encourage late-season activity of the flies. Half-grown maggots were still found in December, and occasionally full-grown maggots, still not in the puparium, were found in January and February. Heavy snow cover protected these late-developing maggots and enabled them to survive temperatures of −10°F.

Table 3.—Hessian fly control with Di-Syston and phorate with resulting grain yields.[a] Columbia, Missouri, 1958–59.

Treatment	Pounds of Toxicant/A.	Wheat Variety	Maggots/100 Plants	Yield (Bu./A.)
Control	—	Vermillion	28.2	34.1
Di-Syston	1	Vermillion	17.2	42.4
Phorate 44D	1	Vermillion	9.5	39.5
Phorate 10% granules	1	Vermillion	1.1	42.5
			L.S.D. 5% =	7.05
Control	—	Knox	46.2	34.6
Phorate seed treatment 44D	0.5	Knox	2.1	35.3

[a] Four replications.

Under this long period of exposure to attack, all insecticides reduced the early infestation but only the 10% phorate granules gave good control over the whole period. Only Di-Syston and phorate granules increased grain yield significantly over the untreated check. In spite of the relatively high infestation of Hessian flies on the Di-Syston plots, grain yields were high and this fact points again to the possibility of collateral treatment effects.

The seed treatment again showed phytotoxic symptoms and, while Hessian fly control was good, no significant increase occurred in grain yield.

The data reported here indicate that practical control of the Hessian fly may be obtained with organophosphate insecticides. One pound per acre of phorate seems to be the minimum dosage level required for reliable control over the necessary protection period, although under optimum conditions lower amounts might be satisfactory. Somewhat greater amounts of Di-Syston and Am. Cyanamid 12008 seem to be needed in order to obtain comparable results. Dosage levels above 1-pound per acre tended to cause phytotoxicity and nullified to an increasing extent the benefits of the treatments. The granulated formulation of phorate seemed to extend the period of protection, probably by extending the residual life of the material also.

It has been found (Brown 1958, unpublished data)

that fertilizer-phorate mixtures fail to give satisfactory control when applied broadcast with a fertilizer spreader before drilling the seed wheat. This loss of control is probably caused by the extreme dispersion of the insecticide in the soil with diminishing probability of its coming into contact with the roots. It seems imperative that the insecticide be applied close to the seed. Presumably the best position is under or around the seed in a band, as has been shown to be the most effective placement for wheat fertilizer. Such positioning could be attained by blending the insecticide with the fertilizer, as has been done in these experiments, or by metering granulated insecticide into the fertilizer stream on the grain drill with equipment such as a grass seeder attachment. This procedure has been used successfully (Bigger 1959, Gehl 1959) and may prove to be the best method of application.

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Fumigation of Buildings to Control the Dry-Wood Termite, *Cryptotermes brevis*[1]

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Fumigation with methyl bromide at a dosage of 2.5 pounds per 1,000 cubic feet for 15 to 19 hours apparently eliminated all natural infestations of the dry-wood termite, *Cryptotermes brevis* (Walker), within 23 of the 24 buildings fumigated. However, considerable differences occurred in mortality among the termites exposed in wooden block cages within these buildings. The mortality in wooden block cages exposed in 18 buildings fumigated with this gas was: 6 buildings 100%, 4 buildings 91% to 99%, 3 buildings 51% to 90%, 3 buildings 11% to 50%, and 2 buildings 0% to 10%. On the other hand, sulfuryl fluoride at a dosage of 2 pounds per 1,000 cubic feet for as little as 1.5 hours killed all the termites in similar blocks. Ethylene dibromide at 2 to 3 pounds per 1,000 cubic feet for 24 hours failed to penetrate the wooden block cages in sufficient quantity to cause any measurable mortality. Furthermore, fumigation with this gas failed to eliminate the natural infestations in two out of five treatments. It is apparent from these tests that sulfuryl fluoride (eight buildings fumigated in tests) was far superior to methyl bromide and methyl bromide was far superior to ethylene dibromide. The use of sulfuryl fluoride should make it possible to greatly reduce exposure time, a great asset in the fumigation of buildings.

The economic damage caused by a dry-wood termite, *Cryptotermes brevis* (Walker), to buildings and furniture in Hawaii amounts to many thousands of dollars annually despite the large sums of money spent in combating it. Spot treatment of infested buildings with insecticides applied as dusts or sprays has often failed to eliminate all the infestations. In recent years fumigation, especially with methyl bromide, has become widely used in Florida and Hawaii against this insect and in California against another species, *Kalotermes minor* (Hagen).

In the spring of 1957, experimental studies were begun on the fumigation of *C. brevis* infested buildings in Hawaii. Since then fumigation tests have been made in 36 buildings (1 of which was refumigated) and these were supplemented with several laboratory tests in fumigation chambers. Three fumigants, methyl bromide, ethylene dibromide, and sulfuryl fluoride, were tested and evaluated. The purpose of this paper is to describe the experimental methods used, show the effectiveness of these gases against this pest and point out pertinent information that may be worthy of consideration in the fumigation of buildings.

ACKNOWLEDGEMENTS.—These fumigation experiments were made possible through the cooperation of a number of individuals in different organizations. Personnel of the Fumiseal Company performed all the regular work involved in the fumigation of the buildings, including most of the gas readings. Mr. Harold Lembright, of the Dow Chemical Company, directed the fumigation and procurement of gas readings in the buildings fumigated with sulfuryl fluoride and the first ones fumigated with methyl bromide. Also, the sulfuryl fluoride was provided by the Dow Chemical Company. Messrs. E. L. Forde, Charles Harvey, and Eugene Yamane, of the Fumiseal Company, not only did or provided for all work actually connected with the fumigation of the buildings, but also contributed helpful suggestions toward improvements in procedure. We are indebted to personnel of the U. S. Department of Agriculture Fruit Fly Laboratory for the extended use of fumigation chambers and especially to Messrs. Stanley Seo and J. W. Balock for technical assistance and suggestions.

Mr. Donald Awai assisted on the termite project during 1957 and made helpful contributions in regard to the development of techniques in handling the termites in the laboratory and the standardization of experimental procedures. He adapted the microscope slide cage as a

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modification of the tongue depressor blade type cage which was being used by Miss Elizabeth McMahan in her biological studies of *C. brevis*. Messrs. Harold Toba and Herman Toma also assisted in carrying out some of the laboratory and bioassay tests.

We are grateful to F. H. Haramoto for the photographs of the cages and to the following companies which provided grant-in-aid funds used in paying the student assistants who worked on the termite research project: California Spray-Chemical Corporation, Dow Chemical Company, Shell Chemical Corporation, and Velsicol Chemical Corporation.

METHODS.—Buildings to be fumigated were wrapped with nylon or polyethylene tarpaulins. Sand snakes,[2] supplemented with small quantities of moist sand, were placed around the bottom of the tarpaulins to hold them tightly against the ground. The usual procedure was to wrap the buildings in the late forenoon or early afternoon, introduce the gas around 4:00 p.m., and remove the tarpaulins between 8:00 and 9:00 a.m. the following day. Prior to sealing, six or more sampling tubes, two hydrothermographs, and several cages of termites were placed in each building. The methyl bromide was heat vaporized and introduced through a hose into the attic above an access opening, which was usually present in the bath room or a closet. A large centrifugal fan directed in an upward position was placed on the floor immediately below the access opening. Sulfuryl fluoride was also introduced through a hose into the attic in a similar manner, except that no heat was used. The ethylene dibromide was poured into a large improvised evaporation pan made of polyethylene tarpaulin, which was placed on the floor. A large fan was directed over the pan to accelerate evaporation of the liquid. Usually the fan was started at the beginning of the introduction of either methyl bromide or sulfuryl fluoride and operated for approximately 2 hours, while with ethylene dibromide the fan was left on until the liquid was evaporated. Dosages were as follows: methyl bromide 2½ pounds, sulfuryl fluoride 2 pounds, and ethylene dibromide 2 to 3 pounds per 1,000 cubic feet. Exposures with methyl bromide and sulfuryl fluoride were for approximately 17 hours and with ethylene dibromide for 24 hours.

Gas readings were taken with either a Gow-Mac or Fumiscope thermoconductivity unit at hourly intervals or less for a minimum of 6 hours immediately following the introduction of either methyl bromide or sulfuryl fluoride. Readings were also taken immediately prior to opening the tarpaulins to begin ventilation at the end of the exposure period.

The termites used in the cages were first extracted from infested wooden products and transferred into gallon jars containing bundles of small pieces of wood that served as shelter and food. As needed for fumigation tests, termites were transferred into petri dishes by gently tapping the pieces of wood over the dishes. Only active apparently healthy individuals were subsequently transferred from the petri dishes into cages for use in the tests. Except for one series of tests only large nymphs were used.

In the tests in buildings, two types of cages were usually used—an open-type and a wooden block-type. The open-type cages used in the earlier experiments were made of a series of five tongue depressor blades bolted together with a section near the center of the middle blade removed to provide a receptacle for the termites (fig. 1, A and B). The gas could readily pass between the blades. A later type was the microscope slide cage (fig. 1, C), which was used as an open-type cage or enclosed within a wooden block (fig. 1, E). The $3\frac{1}{2}'' \times 3\frac{1}{2}'' \times 6''$ or $7''$ wooden blocks were of finished fir selected free of knots and noticeable cracks. Into the center of one end of each block a 1-inch hole was bored to a depth of $4\frac{1}{2}$ inches. This left either $2\frac{1}{2}$ inches or $1\frac{1}{2}$ inches from the bottom of the hole to the end of the block, depending upon whether it was a $7''$ or a $6''$ block. Opposite one of the corners a $\frac{1}{4}$-inch groove was made to facilitate placing the microscope slide cages containing termites into and out of the hole. After either one or two microscope slide cages were inserted, the hole end of the block was made gas tight with a piece of glass sealed in place with melted paraffin (fig. 1, F). In some tests, additional block cages, which had been sealed at both ends in a similar manner, were included.

The standard procedure adopted for the bioassay tests within the buildings was to expose 9 of the $3\frac{1}{2}'' \times 3\frac{1}{2}'' \times 7''$ wooden block cages and 9 open cages, each containing either 10 or 20 large nymphs, within each building and to remove 3 cages of each type at the end of 3 hours, 3 at the end of 6 hours, and 3 at the end of the exposure period. In addition to this usual exposure procedure a considerable number of innovations were made in exposure time and in the area of surface of the wooden block cages made gas tight with paraffin.

Immediately following the removal of the last cages from a fumigated building, all the cages, including three check cages, were opened in the insectary, the condition of the termites recorded, and re-examinations made daily for at least 1 week. Those that received a lethal dose of either sulfuryl fluoride or methyl bromide commonly vomitted profusely (fig. 1, D).

Through the cooperation of the Fruit Fly Laboratory several tests were made to determine the toxicity of methyl bromide to *C. brevis* under controlled conditions within the laboratory. However, these tests were confined primarily to exposures for only 3 hours and at temperatures of 70° F. and 80° F. In the dosage-mortality tests, 15 to 20 large nymphs were used in each petri dish and three of these replicate dishes were placed within each of four 10 cubic foot fumigation chambers. Each dosage was replictaed at least three times. The fumigation chambers were constructed of galvanized sheet metal and the interior surfaces painted. A circulation fan was built into each chamber and operated throughout the exposure period. These chambers had water-sealed covers.

The four chambers were used within a 240-cubic foot constant temperature (±1° F.) chamber. The termites were exposed in the smaller chambers with the covers off for at least 1 hour at the desired temperature immediately prior to the introduction of the gas. The relative humidity in the small chambers was 98% to 100%.

The methyl bromide was introduced through one-hole

[2] Sand snakes are elongate canvas bags approximately 3 inches in diameter and 6 feet in length filled with sand.

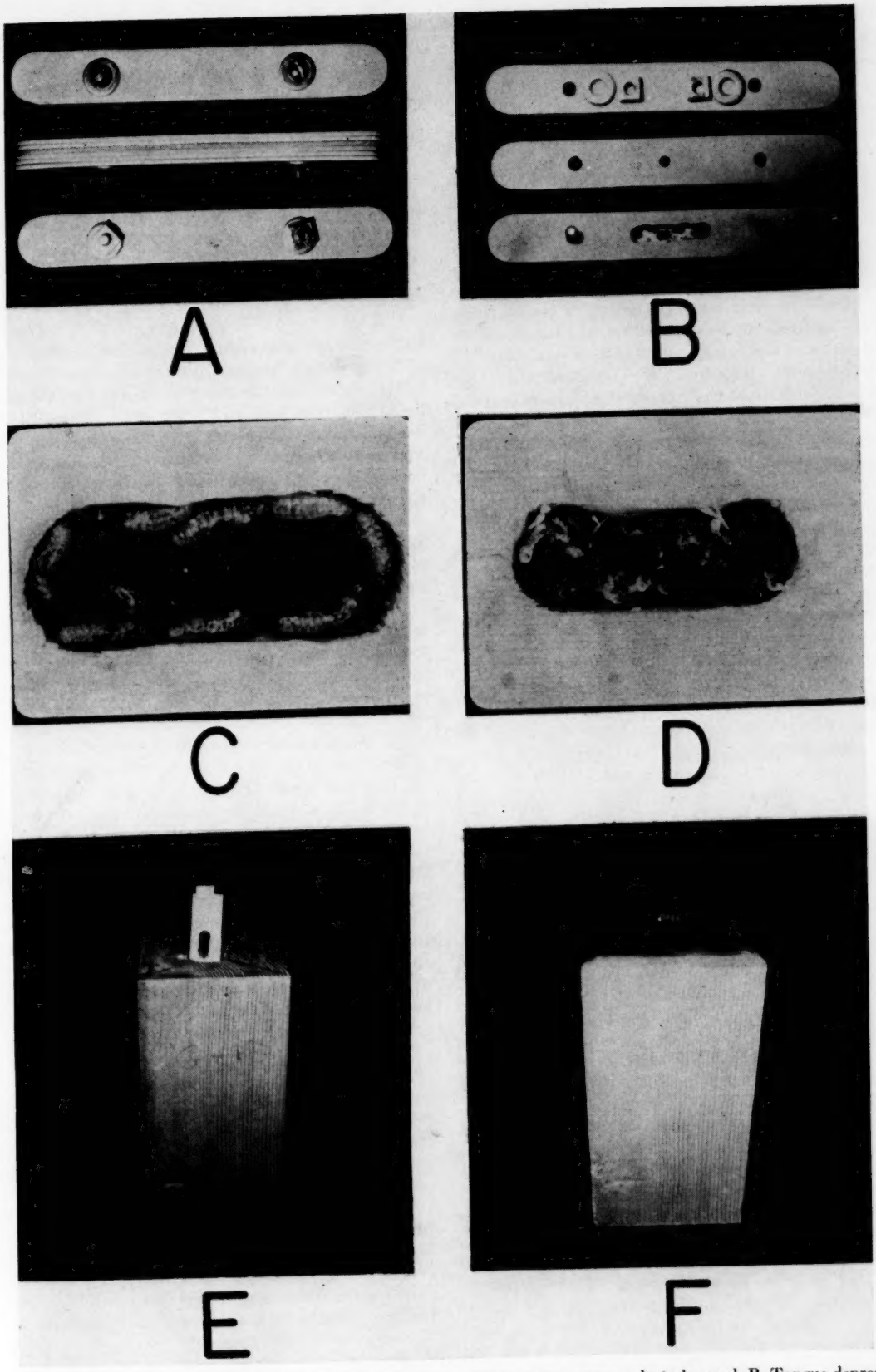

FIG. 1.—Types of cages used in the fumigation studies. A. Tongue depressor type cage ready to be used. B. Tongue depressor type cage opened to show termites in the cavity of the center blade. C. Closeup of termites in microscope slide cage. D. Dead termites after fumigation with methyl bromide. E. A microscope slide cage partially inserted into a $3\frac{1}{2}'' \times 3\frac{1}{2}'' \times 7''$ wooden block cage. F. A 7″ wooden block cage with the open end sealed with a glass plate and paraffin.

rubber stoppers in the covers of three chambers while the fourth chamber served as a check. A 25-ml. burette, accurate to 0.1 ml., specially adapted to deliver the gas under pressure as a liquid, was used in introducing the methyl bromide.

Gas samples were taken immediately prior to the end of the fumigation period and the concentration of methyl bromide determined by chemical analysis as described by Stenger *et al.* (1939). On several occasions, a gas sample was also taken 5 to 10 minutes following introduction to obtain information on the rate of loss in concentration within the chambers. At the end of the exposure period the exhaust fan was turned on, the door opened, and the covers of the small chambers removed. About 30 minutes later the termites were removed from the chambers and taken to the insectary. Pieces of wooden tongue depressor blades and paper toweling were then added to the petri dishes to provide shelter and food. Observations were made daily to determine mortality.

As the experiments progressed, differences in mortality or gas penetration were noted among a few of the wooden block cages. Subsequently, laboratory tests were made to obtain data on individual block differences so that exceptionally "soft" or "hard" blocks could be eliminated. In these tests, approximately 40 large nymphs were inserted into each block cage and the cage sealed with glass and paraffin as described above. The blocks were then placed in a 100-cubic foot fumigation chamber and fumigated with methyl bromide at either 16 or 18 mg./l. for 16½ to 17 hours at a temperature of 80±1° F. The relative humidity was not controlled but ranged between 40% to 60%. The gas was added after a constant temperature in the chamber had been maintained for at least 1 hour. Immediately before the fumigation was completed, a gas sample was taken and analysis for methyl bromide was done by the method described by Stenger *et al.* (1939). After removal from the chamber, the cages containing the termites were removed from the the blocks and kept in the laboratory for daily observation and mortality counts.

RESULTS.—In these fumigation tests, 24 buildings, plus a few detached garages, were fumigated with methyl bromide, 8 with sulfuryl fluoride, and 4 (1 re-treated) with ethylene dibromide. The modifications made in procedure as the program developed, variations in weather such as high winds and rain, and mechanical difficulties with the thermoconductivity units on some jobs made it impractical to attempt to use the fragmentary data obtained in a few of the buildings. Detailed data on gas concentration and bioassay obtained in several of the buildings are presented as illustrative of the intra- and inter-building variations that occurred and generalizations have been made where the data were considered adequate to warrant them.

METHYL BROMIDE.—*Gas Readings.*—Readings obtained from sampling tubes placed at various locations within buildings fumigated with either methyl bromide or sulfuryl fluoride showed that within a short time after the introduction of the gas and the operation of the circulating fan, the concentration was relatively uniform throughout the buildings. This fact is illustrated by the readings obtained in building No. 1-59-1 (table 1) which also indicate the rate of loss in concentration, the buildup in the soil, and the relative uniformity of the gas concentration in the building and in the upper foot of soil 18.5 hours after the gas was introduced.

Table 1.—Gas readings in ounces per 1,000 cubic feet within building No. 1-59-1, which received methyl bromide at a concentration of 40 ounces per 1,000 cubic feet.

Location of Sampling Tube	Hours After Introduction of Gas									
	0.5	1	2	3	4	6	7	8	9	18.5
Peak of roof	38	28	23	23	29	25	23	21	18	10
Top of attic	20	27	23	25	23	21	20	18	16	10
Floor of attic	48	43	33	30	28	26	23	22	19	13
Ceiling of dining room	38	35	36	30	28	27	22	21	17	10
Floor of dining room	38	35	35	29	28	27	23	22	16	10
Ceiling of basement	38	35	36	31	28	27	24	22	17	11
Surface of ground	38	40	36	31	29	27	24	22	17	11
12″ in ground	2	1	6	10	12	11	14	15	15	10

The volume of the above residence was calculated to be 53,000 cubic feet, and 132 pounds of methyl bromide were introduced into the attic through a hose between 1:00 and 1:30 p.m. The circulating fan was then turned on and operated for 3½ hours. The weather was dry and sunny, with a slight breeze. In the dining room the temperature was about 80° F. from 1:30 to 8:00 p.m. and declined gradually to 72° F. at 8:00 a.m. the following day. Throughout the fumigation period the relative humidity within the building was approximately 80%.

Readings were obtained in 18 of the buildings fumigated with methyl bromide at dosages of 2½ pounds per 1,000 cubic feet. In most instances the concentration was around 1½ to 2 pounds per 1,000 cubic feet 1 hour after the gas was introduced, and had dropped below 1 pound by the end of 6 hours. However, the rapidity of loss was much greater in some buildings than in others (fig. 2).

Mortality of Termites Within the Buildings Fumigated.—All the buildings in these experimental tests were known to be infested with dry-wood termites at the time of treatment. Each was carefully inspected at least once within the 15 months following treatment. Living termites were found in the ceiling joists of one of the buildings 15 months after fumigation and the building was refumigated. It was estimated by one firm that living termites were found in possibly 0.5% of the buildings that they had fumigated during the past few years. However, these fumigators are convinced that the procedures they have followed in recent months assure even more reliable results than those obtained previously.

All termites exposed for 6 hours or more within open-type containers or cages in the several experimentally fumigated buildings were killed. Furthermore, the termites were either dead at the time of removal from the buildings or died within 24 hours after removal, indicating that the dosage received was probably in excess of that required to kill them.

The mortality of termites exposed throughout the fumigation period within 7″ wooden block cages in the buildings furnished most of the basic data for evaluation of the relative effectiveness of the treatments. The data obtained from such exposures within 18 buildings fumigated with methyl bromide are summarized below:

No. Buildings	*No. Termites Exposed*	*Per Cent Mortality at End of 4th Day*
6	137	100
4	129	91–99
3	74	51–90
3	84	11–50
2	53	0–10

In another building 20 termites in each of twelve 6″ wooden block cages (240 termites) were all dead within 2 days after fumigation.

Mortality of the termites exposed for 6 hours in 7″ wooden block cages in the 18 buildings was an follows: 3 buildings 76% to 100%, 2 buildings 26% to 75%, and 11 buildings less than 25%. The mortality in the 3-hour wooden block cage exposures exceeded 40% in only one building.

A considerable number of exposures were also made for 14 to 20 hours within 7″ wooden block cages with both ends sealed, but apparently little or no gas reached the termites, for no mortality had occurred by the end of the fourth day. Penetration was apparently more readily accomplished with the grain than across it. Therefore, the gas probably reached the termites in the 7″ wooden block cages by penetrating 2½ inches of wood with the grain.

The average mortality of the termites held in the check block cages was less than 5%, with a maximum of 11%.

Fumigation Chamber Tests.—The dosage-mortality studies on *C. brevis* made within the fumigation chambers showed that the fiducial limits at 95% confidence of the LD_{50} of methyl bromide when used at 70° F. and 80° F. for 3 hours and at a relative humidity of 98% to 100% were 13.6 to 16.4 mg./liter and 13.1 to 13.7 mg./liter, respectively. Probit analysis (Finney 1952) indicated that the LD_{50} points at the two temperatures did not differ significantly. The slopes of the dosage-mortality lines were 9.1 and 10.2, respectively; however, they were not significantly different (fig. 3). As expected, the rate of mortality increased with concentration. It may be seen that mortality caused by methyl bromide occurred by the fourth day (fig. 4).

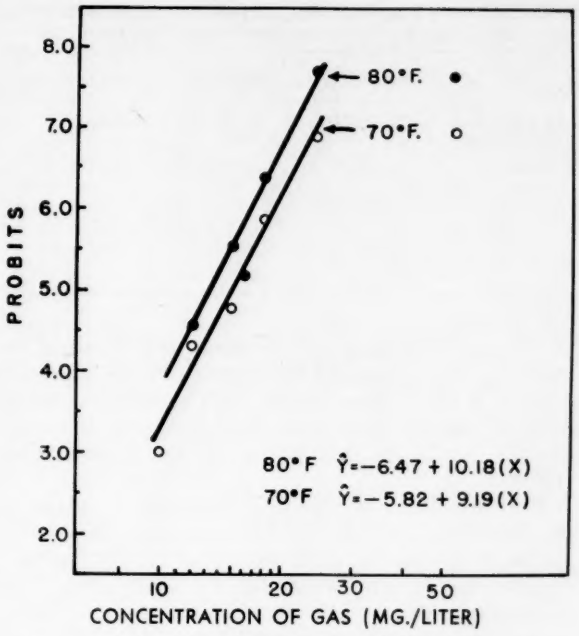

FIG. 3.—Dosage-mortality curve of the effect of methyl bromide on *C. brevis* (3-hour exposures).

Samples taken 5 to 10 minutes after initial introduction of the methyl bromide showed that the concentration in the chamber was 10% to 15% lower than the dosage introduced. In most instances, the loss of gas at the end of 3 hours was between 15% and 20% (table 2). No apparent difference occurred in the per cent of gas loss attributable to the different temperatures.

To determine whether or not differential susceptibility occurred among the stages, small nymphs, large nymphs, and alates were placed in petri dishes and exposed to methyl bromide for a period of 3 hours at a temperature

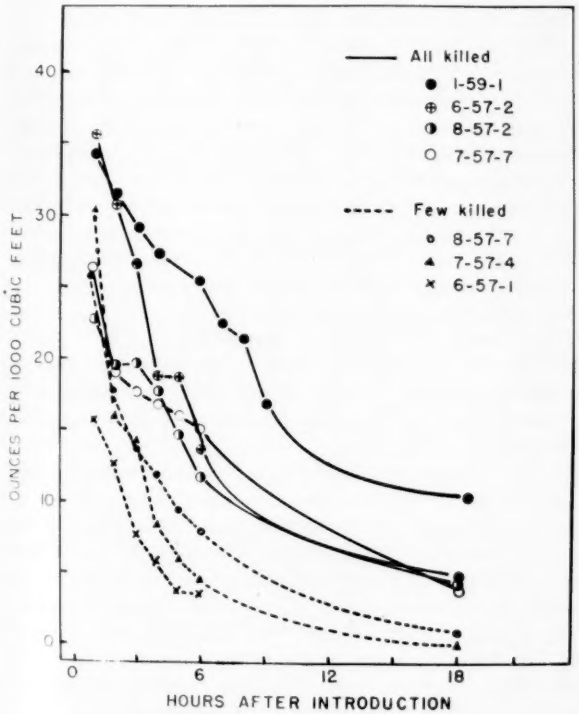

FIG. 2.—The comparison of the retention of methyl bromide in four buildings in which all the termites were killed within 7″ wooden block cages with that of three buildings in which relatively few were killed.

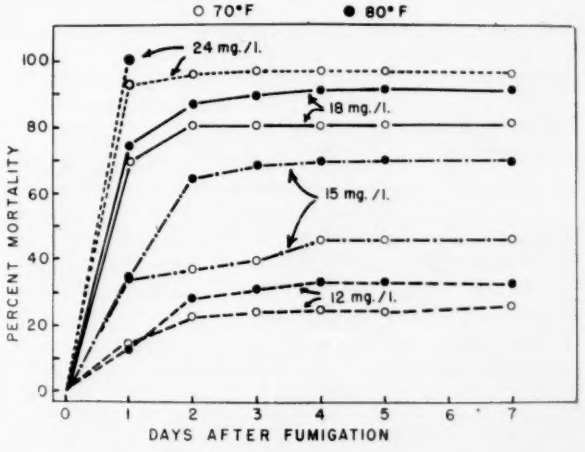

FIG. 4.—Mortality of *C. brevis* following fumigation with methyl bromide for 3 hours at 70° F. and 80° F.

Table 2.—Average per cent gas loss 5 to 10 minutes and 3 hours after introduction of gas into fumigation chambers.

Initial Concentration (Mg./L.)	Per Cent Gas Loss ± Standard Deviation		
	5–10 Min.	3 Hours	
	70° F.	70° F.	80° F.
12	12.5±1.4	13.9±6.7	12.6±7.2
15	—	17.3±3.0	17.1±4.8
18	12.4±2.3	16.9±2.9	17.0±3.4
24	11.2±1.2	15.6±2.6	—

of 80° F., and in another experiment individuals of the three stages were inserted into 7″ wooden block cages and fumigated for 17 hours at 74° F. The results obtained indicated that the alates were more susceptible to methyl bromide fumigation than either small or large nymphs (table 3).

The three series of tests made by exposing several of the block cages within the 100-cubic-foot chamber revealed that three of them were significantly "softer," or more readily penetrated, than the other blocks. The average mortality within the 7″ blocks was also significantly lower than that in the 6″ blocks.

Sulfuryl Fluoride.—*Gas Readings.*—Difficulties were encountered in the operation of the Fumiscope and Gow-Mac machines, which resulted in a paucity of data on some of the sulfuryl fluoride jobs. In one building the average concentration 4 hours after introduction was about 16 ounces per 1,000 cubic feet and at the end of 14 hours it was about 9. Since the dosage used was 2 pounds of sulfuryl fluoride per 1,000 cubic feet, the retention was somewhat better than that in many of the buildings fumigated with methyl bromide. On the other hand, the gas readings indicated a rapid decline in concentration in two of the buildings fumigated with sulfuryl fluoride. In one of these, the concentration was only about 12 at the end of 1 hour, declined to 6 by the end of 3 hours, and to 0 at the end of 6 hours. A booster shot was then introduced, which brought it up to 12 again, but it rapidly declined to 0. In the other building the concentration was about 16 ounces 1 hour after introduction and approximately 5 ounces 3 hours later.

Table 3.—Effect of methyl bromide on the different stages of *C. brevis*.[a]

Type of Fumigation and Cage Used	Dosage (Mg./L.)	Per Cent Mortality		
		Small Nymphs	Large Nymphs	Alates
Open petri dishes (80° F. for 3 hours)	Control	0	0	0
	12	36	44	100
	18	88	92	100
	24	100	100	100
7″ wooden block cages (74° F. for 17 hours)	Control	0	0	0
	12	0	0	37.2
	24	0	0	24.2
	42	23.4	36.6	96.5

[a] Each treatment had 25–30 termites. Fumigation within 10 cubic foot chambers.

Mortality of Termites Within the Buildings Fumigated.—The eight buildings fumigated with sulfuryl fluoride were infested with termites at the time of treatment and no living termites were found after fumigation.

Termites were exposed in each of the buildings in 7″ wooden block cages with one end sealed, and in similar cages with both ends sealed. All individuals exposed in both types of cages for the entire fumigation period died within 24 hours after removal. In these earlier tests usually only two replicates were used.

Supplementary exposures for shorter periods within wooden block cages sealed at only one end and in open-type cages were made in three of these buildings. All (140) termites, 10 in each of 7 wooden block cages and 7 open-type cages, exposed for either 2, 2½, 3, or 5 hours, died within 24 hours after fumigation. Those exposed within block cages for 1½ hours died within 48 hours and those for 1 hour died within 5 days. All those exposed in open-type cages for 1 hour died within 48 hours after fumigation. Some of those exposed for 30 minutes in either block cages or open dishes lived more than 11 days after fumigation.

Ethylene Dibromide.—Two of the four buildings fumigated with ethylene dibromide at a dosage of 2 to 3 pounds per 1,000 cubic feet for 24 hours were found to contain active infestations of dry-wood termites 3 to 4 months after treatment. Furthermore, one of these buildings was re-treated in a similar manner and the infestations persisted after the second treatment. Termites exposed within the 7″ wooden block cages with one end sealed in these buildings appeared to be normal 3 weeks after treatment. However, all termites exposed within open-type cages in these buildings for the entire period (24 hours) of treatment died by the 11th day (fig. 5).

Discussion.—The gas readings obtained at various locations within the buildings before and after stirring of the air and fumigant with the large centrifugal fan showed that the fan was necessary to provide for mixing. However, after thorough mixing had been accomplished there appeared to be no need for further circulation. The gas readings tended to be lower and more variable near the tarpaulin, such as near the peak of the roof, than in the central parts of the buildings. Therefore, it might be postulated that termite infestations near the tarpaulin may have received a somewhat lower dosage of gas than those nearer the center of the buildings. These differences were probably too small to be of practical significance, especially since the temperature was usually considerably higher in the attic than on the first floor or in the basement.

The bioassays based on the data from the block cages did not necessarily give an adequate measure of the effectiveness of the treatments in the eradication of termites from the buildings, for termites within different timbers in the buildings certainly varied in respect to exposure to the fumigants. However, the data suggest that the wooden block cages were probably more difficult to penetrate than the galleries occupied by termites in natural infestations within the buildings. Nevertheless, since all the termites exposed within the wooden block cages were killed in several of the buildings fumigated with methyl bromide, and in all those fumigated with

sulfuryl fluoride, such cages appear to be practical for evaluating the effectiveness of the treatments.

Leakage through the tarpaulins and soil was suspected of being of great importance in some instances. In fact, in those buildings where the loss was especially rapid they were either located on sites of a porous nature, such as sand, rock fill, or cinders, the tarpaulins used showed signs of excessive wear, or there were high winds during the fumigation period. On the other hand, where there was good retention of gas concentration, the buildings were on sites that were not especially porous and the tarpaulins were either relatively new or rain kept the tarpaulins wet. The wetness probably reduced loss of gas through the tarpaulins. Gas retention was generally higher in the larger buildings, which fact might be attributable to the lower surface-volume ratio. Nevertheless, one of the better jobs from the standpoint of gas retention and kill within wooden block cages was a 14,040 cubic foot building (No. 6-57-2, fig. 2). That slow rain and no high winds occurred during the fumigation of this building may be significant. Gas retention was also relatively high in two buildings that were wrapped with new tarpaulins, and one of these buildings was located on cinders. However, the rapidity of buildup of the gas concentration within the cinders under this building (table 1) was less than might have been expected. These findings indicate that leakage through the tarpaulins may be especially important, as previously indicated by Stewart (1957).

Gas loss within the fumigation chambers was probably due mainly to sorption on the walls of the chambers, although there may have been discrepancies in the measurements of the methyl bromide. The sharp drop in concentration, or discrepancy between the introduced dosage and the chemical analysis based on samples drawn 5 minutes after introduction, is perplexing but similar to the results obtained by Sinclair & Lindgren (1952) and others. This result means that the termites in the petri dishes were actually exposed to a lower dosage than that introduced. Therefore, the concentrations used in the dosage-mortality studies were actually lower than those indicated (fig. 3).

The several series of exposures of large nymphs for 3 hours in open petri dishes within the fumigation chambers showed that a concentration of 24 ounces per 1,000 cubic feet at 70° F. did not always give 100% mortality, 16 ounces gave approximately 60%, and 12 ounces seldom killed more than 25%. At 80° F. mortality was not significantly higher than that obtained at 70° F., but more extensive tests at this temperature would probably have shown a significant difference (fig. 4).

The exposures of termites in block cages for 17 hours within the fumigation chambers revealed that a large percentage of them survived at a concentration of 18 ounces of methyl bromide per 1,000 cubic feet, which was the highest concentration used in these tests. On the other hand, in about one-third of the buildings fumigated, all the termites in the wooden block cages were killed. This fact would indicate that a greater dosage (concentration×time) was received by these termites than by those in the fumigation chambers. However, both the initial gas concentration and the temperature during the first 6 hours were higher in these buildings than in the fumigation chambers. These two factors were probably of major importance in the penetration of the wooden block cages, although other factors may also have been important (Sun 1947).

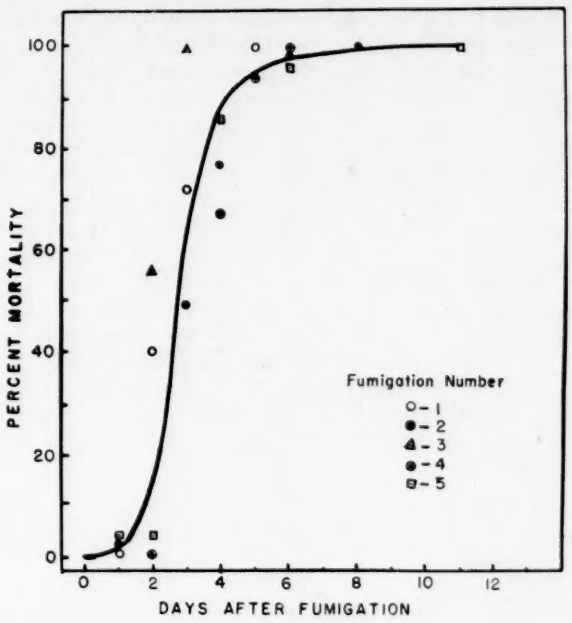

FIG. 5.—Rate of mortality of *C. brevis* exposed in open cages in the four buildings (1 retreated) fumigated with ethylene dibromide for 24 hours.

An important practical aspect in the fumigation of buildings is the exposure time necessary to kill the termites within. During periods of high winds and where business would be interrupted by fumigation, shorter exposure periods would be especially helpful. However, since the mortality obtained from the exposures within the wooden block cages to methyl bromide for 6 hours or less was very low, higher dosages would be needed if the exposure time were reduced. Furthermore, it appears that when the gas concentration drops below 1 pound per 1,000 cubic feet within 3 hours after completion of introduction, which represented about 40% of the initial dosage, additional gas should be introduced to insure satisfactory results (fig. 2). On the other hand, the exposures to sulfuryl fluoride for only 3 hours at a dosage of 2 pounds per 1,000 cubic feet gave excellent results. Since the two gases are similar in toxicity (Kenaga 1957) the sulfuryl fluoride penetrated the wooden blocks far more rapidly and in greater concentration than methyl bromide. This gas also has other advantages over methyl bromide (Kenaga 1957, Stewart 1957). Ethylene dibromide was toxic to termites in open dishes but it failed effectively to penetrate the wooden blocks even with a 24-hour exposure.

These investigations showed conclusively that sulfuryl fluoride at 2 pounds per 1,000 cubic feet was far superior to methyl bromide at 2½ pounds per 1,000 cubic feet in penetrating the wooden block cages and that

ethylene dibromide was ineffective as used. Since methyl bromide effectively penetrated the 7″ wooden block cages in 6 of the 18 buildings fumigated experimentally with this gas but failed to do so in the other 12, a greater dosage (concentration×time) may be justified. Possibly this result could be accomplished without additional gas in most instances, provided sufficient emphasis were placed on the use of *gas-tight* tarpaulins to keep leakage at a minimum. On the other hand, the dosage of sulfuryl fluoride used was apparently in excess of that needed to effectively penetrate the 7″ wooden block cages. With an initial dosage of 2 pounds of sulfuryl fluoride per 1,000 cubic feet the exposure time could probably be safely reduced to 3 hours or less. Further experiments have been planned in which termites will be exposed within wooden block cages to sulfuryl fluoride within buildings to ascertain practical dosages for fumigators to use under Hawaiian conditions.

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Biology of the Oak Timberworm, *Arrhenodes minutus*[1]

W. D. BUCHANAN[2]

ABSTRACT

In Missouri the oak timberworm *Arrhenodes minutus* (Drury) makes "pin holes" that degrade or even destroy the value of potential products obtained from many oak trees. The eggs are laid in blazes and other wounds that expose the bare wood on living trees. The larvae make their galleries across the grain of the wood, and they keep them clean by pushing borings, et cetera, out of them through hair-sized holes made in the wood by the parent females. The life cycle was 2 years for 3 specimens, 3 years for 122, and 4 years for 1 specimen. Pupation takes place near the exit from the larval gallery.

No evidence of attack was found in black and scarlet oak trees that had not been wounded, but attacks were made through wounds in approximately 50% of the trees that were blazed in March, 78% of those blazed in May, 60% of those blazed in June, and 14% of those blazed in late July. In general, wounds less than 3 weeks old seemed to be more attractive sites for egg deposition than older wounds, but in a few instances eggs were laid in wounds that were 2 years old. However, eggs were not laid in wounded trees that were under 5.5 inches in diameter at breast height regardless of the age of the wound.

In Missouri the oak timberworm (*Arrhenodes minutus* (Drury)) (fig. 1) attacks the trunks of many oak trees through blazes and other wounds that expose the wood. "Pin holes" (fig. 2) made in the trees by the larvae (fig. 3) degrade or even destroy the value of potential products of such trees. Because little was known about the life cycle and habits of *A. minutus* in Missouri, a study to gain this information was made on the Pioneer Forest[3] in Dent County, southeast Missouri, during the 5-year period 1954 through 1958. This forest is well managed and contains good stands of second-growth oak-hickory that are better than the average for the Missouri Ozarks.

PROCEDURE.—A total of 864 healthy black and scarlet oak trees was used in this study. The trees ranged from 4.4 to 18.6 inches in diameter at breast height. Each of 18 one-acre plots contained 48 of the trees, distributed at irregular intervals over a distance of 6½ miles. The 48 study trees in each plot were divided into 8 groups, each assigned to 1 of 2 treatments and to 1 of 4 treatment dates.

The two treatments were: (1) Blazes: made with a cruising ax that cut through two or more of the outer growth rings and made a wound 2½ to 3 inches on a side. The chip was removed, leaving the exposed wood more or less smooth. Two such blazes were made on opposite sides of the trunk 3 to 4 feet above the ground. (2) Checks: These trees were not blazed.

All treatments were made in 1954: the middle of March, May, June, and in late July. On each treatment date six trees in each of the 18 plots were blazed and similar trees were left as checks. At various times during the course of the study the trees were examined to find: (a) which trees were attacked, (b) conditions under which attacks were made, and (c) manner in which attacks were made.

ADULTS.—Adults of *A. minutus* were seen in the study plots from early May to late August, but the number was usually greatest from about the middle of May to the

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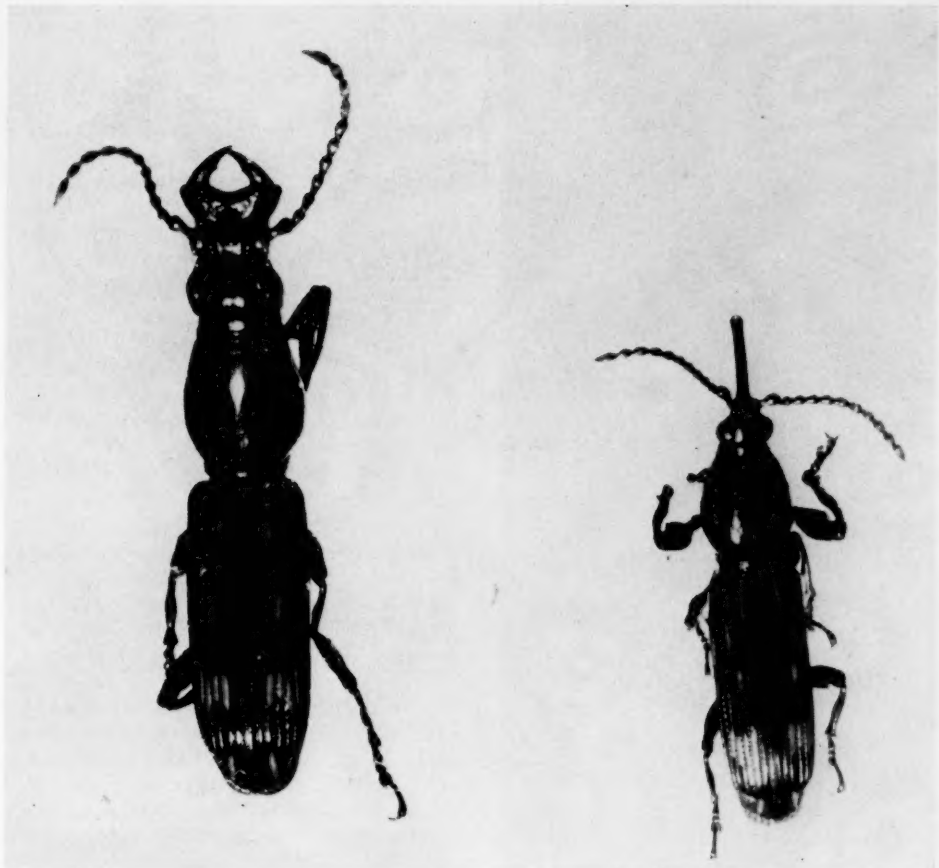

FIG. 1.—Male and female *Arrhenodes minutus* (Drury). The size of the adults varies. The male in this picture was 35 mm. long and the female 25 mm. long.

middle of June. While the population was at its height the sex ratio was about 50–50. But as the population declined, the females decreased more than the males so that after mid-July there were approximately two males to each female.

Both sexes were attracted to fresh wounds, and the females were observed to oviposit in trees through blazes less than 24 hours old. In general the largest number of *A. minutus* was found on wounds that were less than 3 weeks old. However, beetles were seen on older wounds, and in a few instances they oviposited in living trees through wounds that were 2 years old. The smallest tree attacked was 5½ inches d.b.h., and the largest was 18.6 inches, the largest tree that was blazed. Some beetles oviposited in trees that had been dead a year, but they all oviposited in wounds that were made before the tree died. No beetles were found to oviposit in nonwounded dead trees, even though the wood was firm when the bark had fallen or was pried off.

A. minutus was often found in bark crevices on both living and dead trees, but none were found to oviposit, either in nature or under cage conditions, unless the wood was exposed (fig. 4).

The females deposited their eggs in "hair-size" holes that they dug in the wood. Each hole was nearly as deep as the proboscis was long, and the diameter of the hole was only a little larger than the proboscis. The miniature mandibles were used to cut and tear loose tiny wood fibers. These fibers were lifted out of the hole and dropped beside it. Observations made of four females revealed that it took approximately 2 hours to dig a hole. When the hole was satisfactory the female quickly turned around and in a matter of seconds deposited a single egg at the bottom of the hole. No protective cover or fluid was placed over the egg. After an egg was laid, the female became inactive for the first time since she started to dig the hole. The length of time until another hole was started and the total number of eggs laid by a female was not determined. Females disturbed while digging holes did not return to finish them. Sudden movements near either sex caused them to drop to the ground, where they immediately tried to crawl under some protective cover.

EGGS.—Eggs were round, and while not measured were estimated to be less than a millimeter in diameter. At first the eggs were more or less translucent, but they gradually became opaque, and just before they hatched the outline of the larva could be seen inside. The incubation period was not determined, but it appears to be a relatively few days.

LARVAE.—The length of time it took the larvae to complete their development and transform to adults was found by enclosing 48 blazes with cages (fig. 5) made from

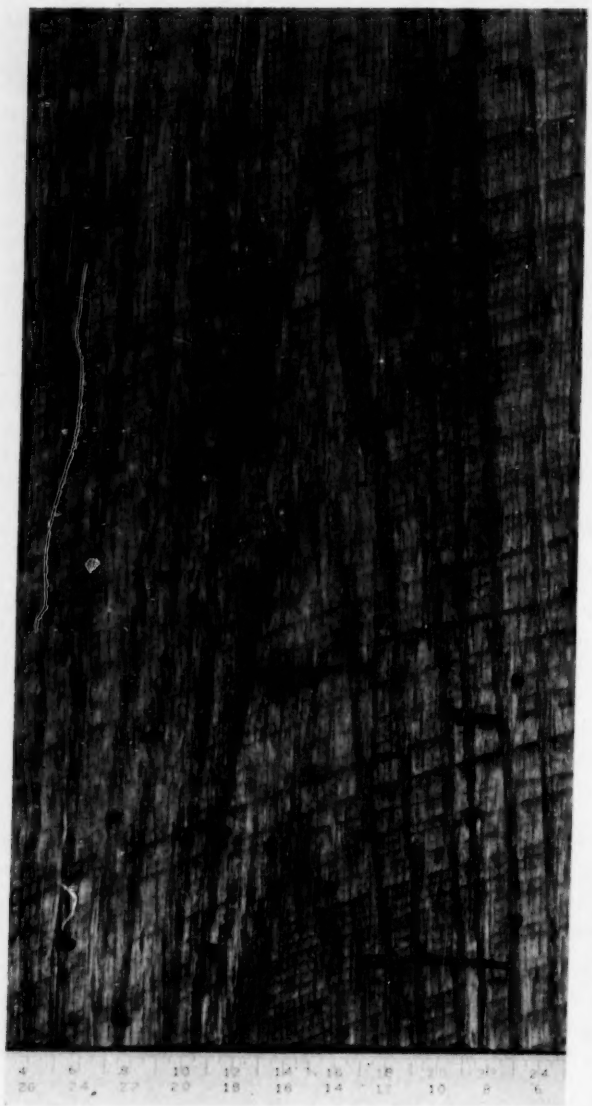

FIG. 2.—"Pin holes" made by the larvae of *A. minutus* made this wood unfit for cooperage.

16- or 32-mesh screen. Adults totaling 126 were reared, and of these 3 took 2 years to complete their life cycle, 122 took 3 years, and 1 took 4 years. All enlarged the hole the parent female made to deposit her egg, and emerged through this hole.

The larval galleries were only slightly larger in diameter than the larvae. The galleries are nearly straight across the grain of the wood with very little slope either up or down. However, galleries that penetrated to within an inch or so of the opposite side of the trunk made a sharp U bend either up or down, and the gallery then went back across the grain of the wood toward the point of origin. The larvae kept their galleries clean by pushing the bor-

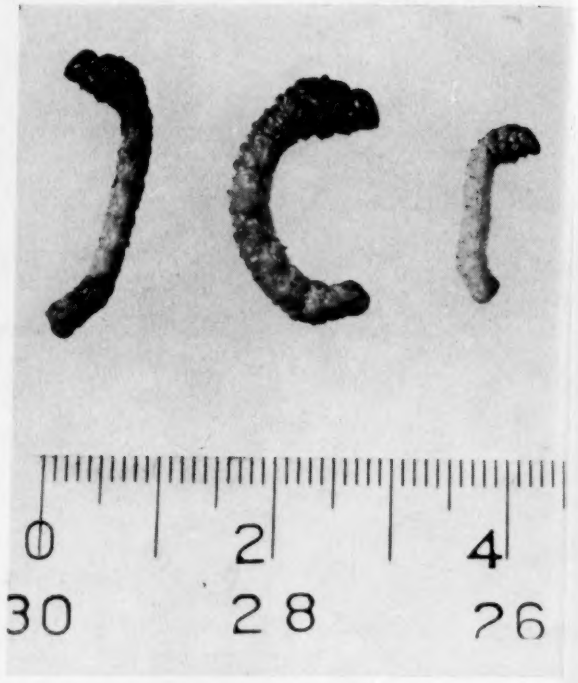

FIG. 3.—*A. minutus* larvae. They are white when first removed from their galleries. The scale is in centimeters, with millimeters as the small divisions.

FIG. 4.—Cage used to confine *A. minutus* adults on unbroken bark on black and scarlet oak trees.

ings (fig. 5) out through the hair-size hole dug by the parent female to deposit her egg. The borings were a dull white and the particles were extremely small. In all cases the galleries were in sound wood, and at no time have they been found to penetrate defective areas.

Throughout this study the presence of borings on the bark or wood was considered proof that larvae of *A. minutus* were in the tree. This conclusion was checked by felling and opening several trees with no borings and others with various amounts. The absence of borings was not always proof that larvae were absent, because heavy rains often washed most, if not all, of the borings away. However, after a few days without rain some correlation between the quantity of borings and the number of larvae seemed to exist. If borings were seen on a tree at any time during the course of the study, that tree was recorded as infested. Borings indicating larval activity were found throughout the growing season, but relatively little evidence of larval activity was observed early in the spring and late fall, and none during the winter.

Using the presence of borings as an index of infestation by *A. minutus*, larvae were present in approximately 50% of the trees that were blazed in March, 78% of those blazed in May, 60% of those blazed in June, 14% of those blazed in late July, and in none of the checks or other undamaged trees.

Most larvae developed in living trees, but a few were found to mature in trees that had been dead 2 years. The bark and wood on these trees were firm and unbroken except for wounds that were made while the trees were alive. No larvae were found in trees that were wounded after the trees had died.

PUPAE.—Pupation takes place near the gallery exit, but the length of the pupal period was not determined.

CONCLUSIONS.—In Missouri *A. minutus* attacked the majority of black and scarlet oak trees through blazes that were made in the spring season on the boles of trees ranging from 5.5 to 18.6 inches in d.b.h. The galleries made by these insects degrade or even destroy the potential value of products that might be obtained from infested trees. No evidence of attack was found in trees that were not wounded. This indicates that *A. minutus* may do little if any injury to black and scarlet oak trees that are not blazed or wounded otherwise so that the bare wood is exposed.

FIG. 5.—Cage enclosing a blaze on a black oak tree. Borings pushed out of the galleries by the larvae may be seen inside and and at the bottom of the cage.

The Effect of Lygus Bug Control on the Yield of Lima Beans[1]

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ABSTRACT

Field tests showed that in years when the tarnished plant bug (*Lygus lineolaris* (P. de B.)) population was high yields of Fordhook 242 and Henderson Bush lima beans could be substantially increased in western New York through the application of insecticides. In addition, plants protected from tarnished plant bug nymphs with insecticide or by screening set more pods earlier in the season and reached maturity earlier than did unprotected plants.

Baker *et al.* (1946) reviewed the literature on lygus bug injury to various seed crops and showed that these insects are capable of causing economic losses to lima beans through direct seed injury (pitting) and pod and blossom drop through feeding and oviposition punctures. More recently other California workers (Middlekauff & Stevenson 1952, Elmore 1955, Stone & Foley 1959) have further explored the effect of these insects on bean production and have established the type and extent of injury caused by *Lygus hesperus* Knight and *L. elisus* Van Duzee.

The tarnished plant bug, *Lygus lineolaris* (P. de B.), is the only species of the genus that occurs in significant numbers on lima beans in New York. Hawley (1922) showed that this species was capable of causing injury to

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developing beans but the importance and extent of injury caused by this insect on lima beans has not been established. Tests were conducted from 1956 through 1959 to determine the effect of the tarnished plant bug on yield of Fordhook and Henderson Bush lima beans in New York.

MATERIALS AND METHODS.—Lima bean seed was treated with lindane and thiram (bis(dimethylthiocarbamoyl)disulfide) and planted in the field in late May or early June. Seed was hand planted in 3-foot rows at a 10-inch spacing for Fordhook 242 and a 5-inch spacing for Henderson Bush lima beans. Normal cultivation was employed to obtain weed control. Insecticide applications were made with a tractor-mounted low-gallonage sprayer equipped with one overhead and two drop nozzles per row. The sprayer was operated at a pressure of 80 p.s.i. and driven to deliver 30 gallons of liquid per acre. Plots were harvested when plants were mature for green bean processing and shelled with a pea viner.

1956 TESTS.—Fordhook 242 lima beans were planted June 5 in eight-row plots 50 feet long. Plots were separated laterally by two buffer rows of lima beans, and 20-foot cultivated alleys separated the four replications. A randomized plot design was used to permit statistical analysis of the results. In this test the insecticides DDT, parathion, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl-dimethyl phosphate, 60% technical) were each applied at the rate of one-half pound per acre. Four applications were made at weekly intervals beginning with the formation of the first blossom buds. Plots were harvested September 18.

The tarnished plant bug was very abundant in the lima bean planting, and an effort was made to evaluate the degree of control achieved with the various insecticides. Such efforts, however, were abandoned when it was found that the sweeping-net method to obtain such data was too injurious to the plants, causing a great number of blossom buds and small pods to be removed. Yield data (table 1) indicate that plots treated with insecticides yielded more beans than untreated plots. Lima beans treated with DDT were noticeably injured by the insecticide or solvent (a 25% xylene emulsifiable preparation was used) and leaf scorch was plainly evident following the third application. This injury may have partly counteracted the beneficial effect of insect control and may explain the smaller increase in yield than in the parathion-treated plots.

1957 TESTS.—During 1957 plots of Fordhook 242 lima beans were set up as in the previous year and the insecticides parathion, Sevin® (1-naphthyl-*N*-methyl carbamate), malathion, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl)phosphorodithioate), and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate) were compared. In addition an identical plot arrangement was established using Green Mountain potatoes as a host plant on which the effectiveness of these insecticides to the insects themselves could be directly measured. A planting of Henderson Bush lima beans was also included. This planting consisted of three plots eight rows wide and 50 feet long replicated four times. Parathion and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) were evaluated on this planting.

Table 1.—The yield of Fordhook 242 lima beans following application of three insecticides to control the tarnished plant bug.[a] 1956.

TOXICANT	RATE (LB./ACRE)	YIELD (LB./ACRE)
DDT	0.5	3,830
Parathion	.5	4,338
Phosdrin	.5	3,884
Check	—	3,476
L.S.D. at 5% level	—	626

[a] Planted June 5; harvested September 18. Treated July 17, 24, 31, August 8.

Table 2.—The yield, pods per plant, and relative maturity of Henderson Bush lima beans following applications of two insecticides to control the tarnished plant bug.[a] 1957.

TOXICANT	RATE (LB./ACRE)	NO. PODS PER PLANT	BLANCHED WHITE (%)	YIELD (LB./ACRE)
Parathion	1.0	46	35	2,562
Trithion	1.0	47	27	2,493
Check	—	45	17	1,922
L.S.D. at 5% level				270

[a] Planted June 5; harvested September 3; treated July 19, 18, 26, August 2.

The tarnished plant bug population on the planting of Fordhook 242 was very light during the entire season. This fact may have been due partly to insect preference for the adjacent plantings of Henderson Bush lima beans and potatoes which supported a moderate tarnished plant bug population throughout the season. In general, however, the tarnished plant bug population was lighter than during the previous season. The Fordhook 242 lima beans were harvested August 28. No differences were obtained in yield regardless of the insecticide applied.

Yield data from the Henderson Bush lima beans harvested September 3 (table 2) show that yields from plots treated with parathion or Trithion were increased significantly above that of the check plots. This increased yield was correlated with a greater maturity in treated than in untreated plants, as indicated by the percentage of seeds that turned white when blanched. Thus the yield differences indicated in table 2 exaggerate those that would have been obtained had each plot been harvested at optimum market grade (about 5% blanching white). Such a harvesting program is difficult, and in these tests an attempt was made to harvest on the basis of optimum maturity in the check plots. Since 17% of the beans from the check plots blanched white (table 2) harvest was 1 to 2 days late for these plots. The number of pods per plant was the same regardless of treatment.

A moderate infestation of the tarnished plant bug was present in the potato planting and spray applications were made July 12, 18, 26, and August 2. Following the spray application on August 2, counts were made on the number of tarnished plant bugs present at 6 hours, 1, 3, and 4 days after application. These data (table 3) indicate that in no instance was control of the tarnished plant bug effective for an extended period. Within 1 day after application the number of tarnished plant bugs collected in 75 sweeps of a 12 inch net had risen to almost 50% of the check population in all but the parathion-treated plots. Limited sweeping and observations indicated that

Table 3.—Control of the tarnished plant bug on potatoes with various insecticides. 1957.

Insecticide[a]	Mean No. Tarnished Plant Bugs Collected in 75 Sweeps of a 12" Sweeping Net			
	Days After Treatment[b]			
	0	1	3	4
Parathion	7	11	15	22
Sevin	4	25	42	47
Malathion	6	18	57	44
Guthion	7	42	46	65
Diazinon	7	25	40	38
Check	49	46	65	53

[a] 1 lb./acre July 12, 18, 26, and August 2.
[b] Treated on morning of August 2. First count afternoon of August 2. Other counts August 3, 5, and 6.

these data obtained on tarnished plant bug control in potatoes fairly represented the control obtained in the lima bean plots. The counts on number of tarnished plant bugs, presented in table 3, include both adult and nymphal stages of the insect. While the sweeping method is not very effective in sampling nymphal populations, it was observed that proportionately fewer nymphs were present in the treated than in the untreated plots.

1958 Tests.—During 1958 plantings of both Fordhook 242 and Henderson Bush lima beans were made to evaluate the effect of tarnished plant bug control on yield. In this season, however, the insect was at a very low population level and no differences in yield were evident.

1959 Tests.—Plantings of Fordhook 242 and Henderson Bush lima beans were made in duplicate on May 27 in eight-row plots 50 feet long replicated four times. Treatments with Sevin, Trithion, and a check were compared in these tests. An additional plot was included for the study of insect abundance throughout the season. Plots were treated at weekly intervals beginning July 8 and four applications were made. Populations of the tarnished plant bug were heavy during the season and the effect of their feeding on the test plots (especially of Henderson Bush lima beans) was evident early in the season. By July 30, plots receiving insecticide application showed a heavy crop of blossoms, while in the untreated plots there was a very light crop of blossoms (fig. 1). However, counts made at this time indicated that adult tarnished plant bug populations were relatively similar (about two per sweep) in both treated and untreated plots. This fact was not true of the immature stages, and because sweeping with a net did not adequately sample the nymphal population, a different method of evaluation was adopted. It consisted of removing 10 consecutive plants from one of the two center rows near the middle of each plot. These plants were carefully cut at ground level and placed in a large pasteboard drum. Any nymphs that fell to the ground during this operation were counted. Chloroform was added to the plants in the drum and the cover closed for about 5 minutes. Then the drum lid was removed and the plants were shaken to remove any insects. The plants were then removed, and the insects and debris remaining in the drum were transferred to a shallow pan for counting. Nymphal counts were made in this way

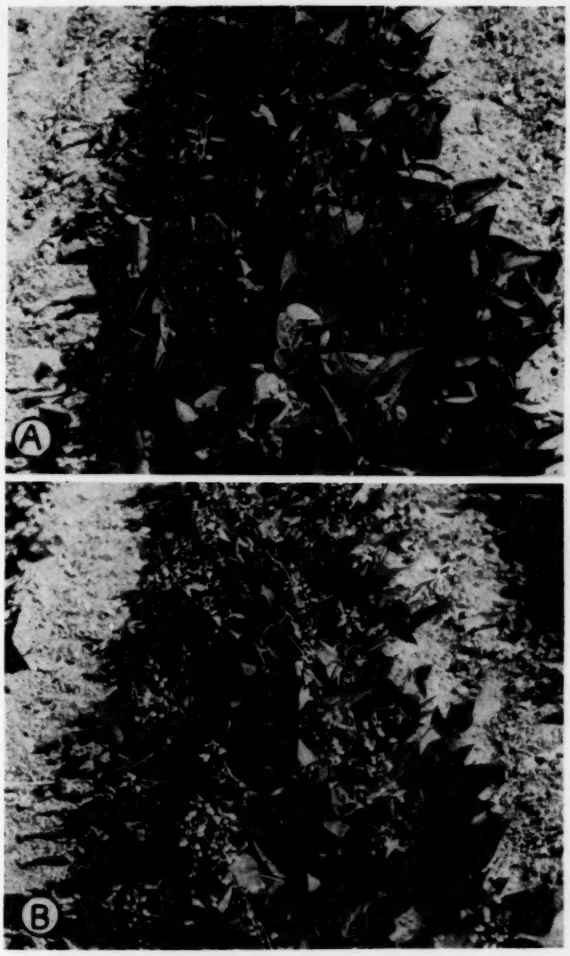

Fig. 1.—Henderson Bush lima beans growing in the field July 30, 1959. A. Untreated plants showing few blossoms; B. Plants treated with insecticide, showing heavy blossoming.

July 30 and August 12, while pod setting was taking place at a rapid rate. These counts (table 4) revealed that both Trithion and Sevin were keeping the nymphal population at a low level in contrast to the check plots. Yield data and counts on the number of pods per plant (table 4) showed that in both varieties of lima bean the high nymphal population was associated with a greatly reduced pod set and yield on untreated plots.

When it was noticed in late July that untreated lima beans were dropping their blossoms without setting pods, nymphs of the tarnished plant bug were the suspected cause. To determine whether or not this premise was true, a series of four screened cages was set up over a previously untreated area on July 28. Each of these cages covered seven plants of Henderson Bush beans. Just prior to installing the cages, the plants were sprayed with Phosdrin at the rate of 1 pound per acre, to remove the insects. Nymphs of the tarnished plant bug were collected from untreated lima beans and 50 were put in each of two of the four cages on July 29, July 30, and August 3. Thus plants in each of two cages were infested with 22 nymphs per plant, while plants in the remaining two cages were

Table 4.—Control of nymphs of the tarnished plant bug and the effect on set of pods and yield of Fordhook 242 and Henderson Bush lima beams. 1959.

Statistic	Date	Treatment[c] Sevin	Trithion	Check
	Fordhook 242[a]			
Nymphs per 10 plants	July 30	5	1	46
	Aug. 13	4	4	27
Pods per 10 plants	Aug. 13	244	267	88
Yield (lbs./acre)		1,978	2,025	946
	Henderson Bush[b]			
Nymphs per 10 plants	July 30	10	2	76
	Aug. 12	5	2	29
Pods per 10 plants	Aug. 12	257	311	39
Yield (lbs./acre)		2,236	2,408	688

[a] Planted May 27; harvested August 21.
[b] Planted May 27; harvested August 26.
[c] Treated with 1 pound actual per acre—July 8, 15, 22, 30.

kept uninfested. The cages were removed August 25 and records made at that time are presented in table 5. These data show that while the number of pods per plant was approximately the same in infested and uninfested cages, plants infested with nymphs of the tarnished plant bug had fewer filled pods, less seeds, and yielded less than the uninfested plants. Inspection of the two cages in which the nymphs had been introduced indicated that only a small percentage had survived more than 15 days. This fact probably explains the uniformity in total pod count. The fact that infested plants had fewer filled pods indicates that the nymphs caused a delay in pod setting by the infested plants.

DISCUSSION.—The results obtained in the present study show that during years when the tarnished plant bug is abundant, nymphs of this species may cause considerable loss in yields of green lima beans of both the Fordhook 242 and Henderson Bush varieties. When the population of this insect is low, little economic loss may result, but in heavy infestations the green yield of Fordhook 242 lima beans may be reduced as much as 50% and of Henderson Bush beans by 70%, as indicated by results of 1959 experiments. Yield reductions are due primarily to a shedding of the blossoms and newly set pods early in the season. This effect may be partly overcome in some years by the ability of the plant to set additional pods as the season advances. In such cases beans are less uniform in maturity, and harvest may be so delayed that the crop is ruined by an early frost.

Table 5.—Mean number of pods, filled pods, seeds produced, and weight of seeds of Henderson Bush lima beans infested and not infested with tarnished plant bug nymphs in field cages.[a] 1959.

Statistic	Cage No. 1	2	3	4
Nymphs[b]	+	+	−	−
Mean total pods per plant	29	34	30	36
Mean filled pods per plant	5	7	11	13
Mean number seeds per plant	12	16	29	30
Mean weight of seeds per plant (gm.)	9	9	18	15

[a] Seven plants in each cage.
[b] Nymphs of tarnished plant bug, 50 per cage on July 29, 30, August 3.

During the course of this work the authors saw little evidence of direct seed injury caused by lygus bug feeding, as was reported from California for *L. hesperus* Knight and *L. elisus* Van Duzee. It may be that this seed injury occurs under some situations because of tarnished plant bug feeding, but the most obvious effect noted in the present work was the shedding of blossoms and pods during the early part of the period during which the lima beans normally set pods.

The work reported here indicates the necessity for controlling the tarnished plant bug if full yield potential of lima beans is to be realized in New York. The minimum number of applications necessary for satisfactory control has not been determined, but two or three may be adequate. It is recommended that insecticides for tarnished plant bug control be applied as soon as the first blossom buds form and that applications be repeated until an adequate set of pods has been obtained.

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European Corn Borer Populations in Relation to the Estimation of Crop Loss[1]

H. C. Chiang, F. G. Holdaway, T. A. Brindley and C. R. Neiswander[2]

Abstract

Studies were conducted at Ankeny, Iowa; Waseca, Minnesota; and Wooster, Ohio, during 1953, 1954, and 1955 to analyze the relationship between midsummer and fall European corn borer (*Pyrausta nubilalis* (Hbn.)) populations and their effect on corn yield. These relationships are affected by the interactions of a number of factors: (1) the proportion of the first brood that pupate in the summer, (2) the selection of fields and plants for egg deposition by the moths, and (3) the survival of the different developmental stages of the second brood individuals. A summer population of a given size may give rise to fall populations manyfold larger, or may end up with fall populations a fraction of the original size. Furthermore, fall populations of the same size may consist of very different proportions of the residual first-brood population and the second-brood population.

The quantitative relationships between midsummer and fall populations are further complicated by the following factors: (1) Differential egg deposition owing to plant development; the plants that were more attractive to moths for egg deposition during the first brood will be less so during the second brood, and vice versa. (2) Nonattractiveness to the second-brood moths for egg deposition of the plants that are heavily infested with first-brood borer. (3) Decreased survival of the second-brood larvae on plants with heavy first-brood injury. This fact may have been the result of a lack of suitable feeding sites on such plants.

The paradoxical relationship between borer population and yield as shown in some cases, *e.g.*, the fewer borers on the plants the less the yield, or the more borers the greater the yield, is clarified on the basis of the foregoing analyses of the borer population dynamics.

Based upon these analyses the conclusion is reached that while the fall population may be a reliable basis for estimating the borer population entering hibernation, the summer population is probably a more realistic index for estimating the loss in yield of corn caused by borer infestation in the current year.

One of the important considerations in controlling an insect is the economic gain—the portion of the crop loss owing to the infestation that is expected to be saved by a control practice minus the expenses of the control practice itself. Thus the accurate estimation of the crop loss caused by the infestation is an important aspect of economic entomology. In the case of the European corn borer, *Pyrausta nubilalis* (Hbn.), considerable difficulty has been encountered in the North Central region in estimating the loss of corn yield owing to borer injury.

A reliable method for estimating yield loss caused by corn borer attack depends on an adequate knowledge of the relationships between borer populations and plant growth. Details of these relationships have been discussed elsewhere (Chiang & Holdaway 1959). Some aspects of the borer population dynamics, which not only are interesting ecologically but also have a bearing on the practical problem of the estimation of crop loss, will be discussed in this paper.

Literature Review.—A considerable number of reports have been published on various aspects of the population changes of the European corn borer in various areas. The effects of environmental factors on borer populations in general were studied by Huber *et al.* (1928), and on the moth population in particular by Stirrett (1938). Since the egg population in a particular field determines the initial level of infestation, the causes of the variation in egg population have received considerable attention. The effect of the growth stage of the host plant on egg population in both one-brood and two-brood areas has been studied by, among others, Neiswander & Huber (1929), Schlosberg & Mathes (1937), Patch (1942), Beard (1943), and Chiang & Hodson (1950). They all concluded that more advanced corn will receive more first-brood eggs. Patch (1942) further showed that this differential is less marked in areas where the general level of corn height is high. The relative abundance of first- and second-brood borers as affected by the date of planting was discussed by Hibbs (1953).

The effect of the growth stages of the host on larval population was also studied by various authors, such as Hervey & Hartzell (1931) and Kelsheimer & Polivka (1931). They showed that there is an optimum stage of plant growth for borer survival. The quantitative relationship between the egg population and the larval population has been studied by Chiang & Hodson (1952).

The relationship between borer population, crop loss, and stalk breakage was studied by Patch *et al.* (1941) in a single-brood area. They showed that the borer produced, on an average, 3% loss in yield per borer per stalk. But later Patch *et al.* (1942) reported that the reduction in yield owing to borer infestation may be affected by factors such as the normal yielding capacity of the variety, the weather, the soil fertility, et cetera. The effect of second-brood borers on yield was studied by Deay *et al.* (1949), and by Chiang *et al.* (1954). The relative importance of first- and second-brood borers on yield and on stalk breakage was studied by Chiang & Hodson (1950).

Certain aspects of the quantitative relationship between the infestations of the two broods were analyzed by Chiang & Hodson (1959) for a 10-year period based on results of one field each year. Some additional aspects of these relationships are critically analyzed in the present study, which was carried out concurrently at three widely scattered localities in the corn-growing area of the north-central region of the United States. The latter feature

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adds much value to the results of the study.

BASIC RELATIONSHIPS BETWEEN THE MID-SUMMER AND FALL POPULATIONS.—The life cycle of the European corn borer in the north central region has been discussed in detail by Everett *et al.* (1958), and the basic relationships between the infestations of the two broods by Chiang & Hodson (1959). For the purpose of the present discussion a summary of some pertinent aspects will be given in the following paragraphs.

The borer overwinters as a full-grown larva. The larvae pupate in the spring and the moths emerge in the late spring and early summer. Eggs are deposited shortly after on the leaves of corn plants. The larvae feed first on the portions of the leaves that are still in the whorl. Later they feed in the leaf sheaths, ribs, and then burrow into the stalks. Some of the larvae pupate in midsummer while others remain as larvae throughout the fall and winter. The later group, while constituting part of the overwintering population, represents the residual population from the first brood. The larvae that pupate in midsummer give rise to a second brood of moths. These moths also deposit eggs on the corn plants, which are then at their maximum height with leaves fully extended. The larvae feed first on plant tissues near the leaf axil where pollen grains are often accumulated, or seek shelter and feed in between the husks and around the silk of the ear. They begin to bore into the stalks at an earlier age than do the first-brood larvae. Those in between the ear husks may enter the inner portions of the ear, the kernels, cob, and shank. In certain parts of the north-central region not all these larvae reach maturity before the winter. The immature larvae are killed by the frost during the fall or by shortage of suitable food if the plants are killed or mature and dry before the borers have completed their feeding, while those that reach maturity will have a good chance of overwintering successfully.

From the life cycle of the corn borer as described above it becomes apparent that at two times during the year the borer populations are made up principally of full-grown larvae, in the midsummer and in the late fall. The size of the borer populations in a given field may be very different at these two seasons. The quantitative relationship between the first and the second broods of corn borer in a general area represents a rather complex type of population dynamics. This relationship is affected by interactions of a number of factors, such as (1) the proportion of the first brood that pupated in the summer, (2) the selection of fields and plants for egg deposition by the second-brood moths, and (3) the survival of the different developmental stages of the second-brood individuals.

A high proportion of summer pupation increases the second-brood moth population. Thus in general it results in a fall population that is higher than the summer population. But a high proportion of summer pupation also depletes the residual first-brood borers. Thus when the survival of the second-brood borers is exceptionally low, a high summer pupation may result in a fall population which is lower than the summer population. Thus a summer population of a given size may give rise to fall populations manyfold larger, or may end up with fall populations a fraction of the size of the summer population. Furthermore, fall populations of the same size may consist of very different proportions of the residual first-brood population and the second-brood population because of the variable proportions of summer pupation and the variable levels of the survival of second brood larvae.

FACTORS COMPLICATING THE BASIC RELATIONSHIPS.—Besides the basic relationships discussed in the previous section, there are three factors that may further complicate the quantitative relationships between the borer population during the summer and that during the fall: (1) the differential egg deposition owing to differences in stages of plant development, (2) nonattractiveness for egg deposition caused by other differences in plant conditions, and (3) the unfavorable effect of the first-brood infestation on the survival of the second-brood borers. As a result of these factors, fields or plants having heavy infestation of the first brood are not likely to have heavy second-brood infestation, and vice versa. The operation of these factors will be discussed in the following paragraphs.

1. *Differential Egg Deposition Owing to Plant Development.*—European corn borer moths prefer, for egg deposition, plants that are most vigorous at the time. The first-brood moths prefer the taller corn and the second-brood moths prefer the later and more succulent corn. The plants that were taller during the first-brood egg deposition are more advanced toward maturity and less succulent during the second-brood egg deposition. The plants that are succulent during the second-brood egg deposition were probably too young and too short to receive many egg masses during the first brood. Thus in a general area the first-brood infestation would be more concentrated in earlier planted fields, and the second-brood infestation would be more concentrated in the later-planted fields.

This phenomenon of differential egg deposition of the two broods has been reported by many workers. But intensive records were obtained in the plots of this study in which two single crosses of corn were used, Oh43×Oh51A, which is borer resistant, and WF9×M14, which is borer susceptible. Two plantings were made, one early and one late within the period of farm planting in the areas. The study was carried on concurrently at Ankeny, Iowa; Waseca, Minnesota; and Wooster, Ohio.

The data on the amount of egg deposition in the different plantings secured in 1953, 1954, and 1955 are of interest. Random plants were checked for egg masses during the period of active egg deposition for both broods. The number of egg masses observed in the four plantings are shown in table 1.

These data show that the egg deposition during first brood was always higher in the early planting than in the late planting of the same variety, and that egg deposition during the second brood was higher in the late planting than in the early planting of the same variety. In other words, the planting which was more attractive to moths for egg deposition during the first brood was less so during the second brood, and vice versa.

The effect of the above relationship on the borer populations in the fall may now be examined. In a given season when the summer pupation is high, the early plantings are likely to show a reduced fall population when compared with the midsummer population, while the late plantings are likely to show an increase. Also, if the summer pupation is low, the late planting will be relatively

Table 1.—Egg deposition by the first- and second-brood moths on corn of different dates of planting.[a]

Station	Year	Brood	Oh43×Oh51A (R.) Early	Oh43×Oh51A (R.) Late	WF9×M14 (S.) Early	WF9×M14 (S.) Late
Ankeny	1953	1st	83	0	81	2
		2nd	—	—	—	—
	1954	1st	278	61	178	6
		2nd	212	269	156	267
	1955	1st	378	26	385	26
		2nd	58	67	25	133
Waseca	1953	1st	33	23	19	9
		2nd	185	308	225	234
	1954	1st	58	29	69	17
		2nd	202	225	123	158
	1955	1st	4	0	0	0
		2nd	33	48	37	48
Wooster	1953	1st	96	69	125	71
		2nd	—	—	—	—
	1954	1st	2	0	2	0
		2nd	—	—	—	—
	1955	1st	0	0	4	0
		2nd	0	32	0	17

[a] Number of egg masses per 100 plants.

free of borer population caused by a low moth population, and the earliest planting will have a considerable population during the fall, which is made of the high residual population of first-brood borers.

2. *Nonattractiveness in Egg Deposition Owing to Plant Conditions Other Than Growth.*—Besides the factor of synchronization of plant development to the activity of egg deposition of the moths, the physical condition of the plants is also important. Some data were collected at Waseca, Minnesota, during the 1954 season.

In the NC-20 study plots four levels of first-brood borer infestations were maintained. The four levels of infestation, as designated by treatments, were:

Treatment 1. Kept free of borer infestation by spraying (with EPN wettable powder at ½ to ¾ pound actual per acre) three to four times during the first-brood egg hatching period.

Treatment 2. Natural amount of borer infestation prevailing during the season.

Treatment 3. Natural infestation augmented by three egg masses per plant. The egg masses were produced in the laboratory and were placed on the plants artificially.

Treatment 4. Natural infestation augmented by six egg masses per plant.

Plants in these plots harbored different numbers of first-brood borers at the midsummer, and suffered different degrees of injury. The egg deposition by the second-brood moths on plants of these treatments were checked in the Waseca plots in 1954, and is shown in table 2, along with the borer populations at midsummer. Only the data from the early susceptible planting are presented; the other plantings showed similar trends.

These data show a definite nonattractiveness of the plants that are heavily infested with first-brood borers, to the second-brood moths for egg deposition. The heavily infested plants suffered a great deal of injury: feeding holes, tearing of the leaf blades, and breakage at the leaf ligules. The nonattractiveness of these plants to moths for egg deposition may have been caused merely by a lack of a suitable surface. This, of course, should not exclude the possibility of other factors, such as premature ripening of the plants owing to borer injury.

This type of nonattractiveness again has an important bearing on the population dynamics of the borers. When the per cent of summer pupation is high, plants that escaped first-brood infestation are more heavily infested with second-brood borers; hence they might have a fall population higher than the summer population. The plants heavily infested with first-brood borers, however, are likely to have a fall population lower than the summer population because of summer pupation, natural mortality of first-brood borers, and failure to attract second brood oviposition.

3. *Effect of First-Brood Injury to Corn Plants on the Survival of Second-Brood Larvae.*—Studies were conducted at Waseca, Minnesota, to determine the effect of the first-brood infestation on the survival of second-brood larvae. A group of plants in the early planted WF9×M14 were artificially infested with three egg masses per plant during the first-brood, and again three masses per plant during the second brood. A second group of plants in the same planting were infested with three egg masses per plant during the first brood and six egg masses per plant during the second brood. Within the study plot itself, there were plots which were sprayed during the first brood and hence were practically free of first-brood infestation, but were artificially infested with three or six egg masses per plant during the second brood. Thus these plots offered four different combinations of first- and second-brood borer populations, as designated in columns 2 and 3 of table 3.

By comparing the second-brood larval populations in treatments 6 and 9–6, or treatments 7 and 9–7, the effect of the presence and absence of first-brood infestations on the survival of the second-brood borers at two levels of population density may be observed.

Twenty-four plants were dissected in each of treatment

Table 2.—Borer population in midsummer and the egg deposition during the second brood.[a]

Criteria (No.)	Treatment Number 1 Borer Free	2 Natural Infestation	3 Natural Infestation plus 3 Egg Masses	4 Natural Infestation plus 6 Egg Masses
Borers per 100 plants at midsummer	0	47	519	608
Second-brood egg masses per 100 plants	150	83	50	33

[a] Data taken at Waseca, Minnesota, 1954, in the early planted WF 9×M14 of the NC-20 plots.

Table 3.—Number of first- and second-brood borers in plots of different combinations of the two broods of borer populations.[a]

Treatment No.	Infestation		Number of Borers per Plant	
	1st Brood	2nd Brood[b]	1st Brood	2nd Brood
6	None or negligible	3 egg masses	0.1	5.3
9-6	Nat. inf. plus 3 egg masses per plant	3 egg masses	5.4	1.6
7	None or negligible	6 egg masses	0	6.6
9-7	Nat. inf. plus 3 egg masses per plant	6 egg masses	4.9	3.9

[a] Data taken at Waseca, Minnesota, 1956, in the early planted WF9×M14.
[b] Natural infestation plus number of egg masses per plant as indicated.

6 and 7, and 12 plants were dissected in each of treatment 9–6 and 9–7, about 1 week after the artificial second-brood infestations were made in these plots. At that time the youngest first-brood larvae were in the fourth instar, and the oldest of the second-brood larvae were in the third instar. Thus the larvae found on the plants in the different plots could be designated as either the first or the second brood. The results are shown in table 3.

These data show that on the plants where the first-brood infestation was heavy (treatments 9–6 and 9–7) the survival of the second-brood larvae was reduced considerably. This finding was further confirmed in 1957. One possible explanation of this fact may be offered on the basis of some field observations. Under severe infestations most of the leaf axils, where the young second-brood larvae usually occur and feed, were injured and had deteriorated, owing to the feeding of the first-brood larvae, and thus had become unsuitable for feeding by the second-brood larvae.

Combining the results of this section with those of the previous section, it is clear that heavy first-brood infestation is correlated not only with a low egg deposition by the second-brood moths, but also with a low survival of the larvae that did hatch on the plants.

COMBINED EFFECT OF THE ABOVE FACTORS.—As discussed earlier, borer populations in the midsummer consist of only the first-brood borers, while those in the fall consist of various proportions of the residual first-brood population and the second-brood population. These varied proportions were shown to be caused by factors such as the per cent of summer pupation, the amount of second brood egg deposition, and the survival of the second-brood larvae.

One particular set of records secured in the NC-20 study plots show the combined effect of these factors on the relationships between the midsummer and fall populations. Data on the borer populations in midsummer and in the fall have been taken at all three stations and in all the plantings. For the purpose of the present discussion, only the data taken on the early planted susceptible WF9×M14 will be presented, as in table 4.

These data show two points illustrating the interactions of the factors discussed previously.

1. In the plots where the first-brood infestation was low (natural infestation—treatment 2) an increase in the population commonly occurred during the fall (compare columns A and C). This increment was caused by the second-brood borers. But since some of the first-brood borers have pupated, hence will not appear in the fall population, the portion of the population observed in the fall that was contributed by the second brood was probably higher than the difference between the two population counts. An exceptional situation was shown in the 1955 Ankeny records. This situation will be discussed presently. In the plots where the first-brood infestation was higher (treatment 4—natural infestation plus six egg masses per plant) decrease in the fall population occurred (compare columns B and D). This decrease was likely owing to the fact that some of the first-brood borers present in the midsummer had pupated, yet the amount of second brood that had been added to the population was not large enough to compensate for the decrease in the first-brood borer caused by pupation and natural mortality, a fact clearly shown in the previous section. The Ankeny 1955 records mentioned earlier showed a decrease in the fall in the naturally infested plots. Since the natural population was quite high, the same factors operating in treatment 4 might be operating in this case.

2. At midsummer, the borer populations were, as would be expected, a great deal higher in the plots of treatment 4 (natural infestation plus six egg masses) than in the plots of treatment 2 (natural infestation only). This differential was true at all stations and in all the years. In the fall, however, one group of records (Wooster 1953, 1954, and 1955 and Waseca 1953 and 1954) showed, as in the summer, that more borers occurred in treatment 4 than in treatment 2, while the other group (Ankeny 1953, 1954 and 1955, and Waseca 1955) showed the reverse, *i.e.*, the fall population was lower in treatment 4 than in treatment 2. An explanation of this difference in the trend of population changes in the two groups of records follows:

At Ankeny in all the 3 years and at Waseca in 1955 the summer pupation was close to 100%. Thus practically no first-brood borers were carried over to the fall. As a result, the plots of treatment 4 were as completely deprived of borer population as the plots of treatment 2 at the beginning of the second brood. Thus even though treatment 4 had a higher first-brood population than treatment 2, the size of the population in the fall was determined solely

Table 4.—Midsummer and fall populations of borers at two levels of first-brood infestation in the early planted susceptible hybrid.

Station	Year	Midsummer Population[a] (borers/100 plants)		Fall Population[a] (borers/100 plants)	
		Treatment 2	Treatment 4	Treatment 2	Treatment 4
		A	B	C	D
Ankeny	1953	11	83	90	62
	1954	240	686	716	490
	1955	167	250	136	57
Waseca	1953	3	342	115	118
	1954	47	608	65	127
	1955	0	100	72	42
Wooster	1953	72	306	70	148
	1954	0	197	60	120
	1955	3	139	62	72

[a] Treatment 2 consisted of natural infestation; treatment 4 consisted of natural infestation plus six first-brood egg masses.

Table 5.—The yield and fall population in early planted plots with two different levels of borer infestation and in early and late planted plots with only natural infestation of both broods.[a]

		Treatment 2		Treatment 4					
		Natural Borer Infestation		Natural Infestation plus 6 Egg Masses		Early Planting[b]		Late Planting[b]	
Station	Year	Yield (bu./A.)	Fall Population (No. of Borers/100 Plants)	Yield (bu./A.)	Fall Population (No. of Borers/100 Plants)	Yield (bu./A.)	Fall Population (No. of Borers/100 Plants)	Yield (bu./A.)	Fall Population (No. of Borers/100 Plants)
Ankeny	1953	*103*	*90*	*99*	*62*	103	90	95	333
	1954	*96*	*716*	*79*	*490*	*96*	*716*	*104*	*1,174*
	1955	*84*	*136*	*72*	*57*	*84*	*136*	*92*	*214*
Waseca	1953	114	115	110	118	*114*	*115*	*117*	*141*
	1954	95	65	74	127	*95*	*65*	*83*	*40*
	1955	*98*	*72*	*85*	*38*	*98*	*72*	*73*	*66*
Wooster	1953	72	70	59	148	73	70	65	345
	1954	66	60	64	120	*67*	*60*	*60*	*40*
	1955	88	62	77	72	88	62	89	62

[a] All plots were planted with susceptible WF9×M14.
[b] All plots received the natural borer infestation of both broods.

by the size of the second-brood population. Since the plants in treatment 2 were much less severely injured than those in treatment 4, there would have been more eggs deposited and a higher survival of the larvae, and hence more second-brood larvae building up the fall population in treatment 2 than in treatment 4.

At Waseca in 1953 and 1954, however, the summer pupation was only about 50%. The remaining borers were carried over as the residual population. The residual first-brood population was much higher in treatment 4 than in treatment 2. Thus even though more second-brood eggs were deposited and more second brood-larvae developed in treatment 2 than in treatment 4, the summer residual population contributed enough to make the fall population larger in treatment 4 than in treatment 2.

Significance of the Relationship Between Summer and Fall Populations to the Estimation of Crop Loss.—In the north-central region the number of borers present during the fall is used as the basis for estimating the loss in corn yield owing to borer damage. Chiang & Hodson (1950) showed, however, that in Minnesota only the first-brood borer infestation reduces the growth of the corn ears, and thus directly reduces the yield, while the second-brood infestation increases stalk breakage, which may reduce the efficiency of machine harvesting, but does not reduce the ear growth.

The yields of treatments 2 and 4 in the NC-20 study plots are given along with the fall population of borers in table 5. These data show that the yield was invariably lower in treatment 4 than in treatment 2, as would be expected on the basis of the high first-brood borer infestation in treatment 4. It is noteworthy that at Ankeny, in 1953, 1954 and 1955 and at Waseca, in 1955 (italicized records), the borer population in the fall was smaller in treatment 4 than in treatment 2. If estimates were made on the basis of the fall population, the crop loss in these plantings would be lower, and hence the net yield would be higher in treatment 4 than in treatment 2. This premise would be contrary to the actual results on yields. Thus when records of the fall population and yield are considered, these particular results would lead to the impression that *the less borers the less yield, or the more borers the greater yield.* This apparent paradox can be clarified by recognizing the significance of the components of the fall population in respect to the broods.

Similar paradoxical results may be found when one compares the yield and the fall population of plots of different dates of planting. Table 5 shows the yield in the early- and late-planted plots of WF9×M14 along with the fall borer populations in these plots. All the plots included were subjected only to natural borer infestation during both broods (treatment 2).

Comparison of the results of the early and late plantings at Ankeny, in 1954 and 1955; at Waseca, in 1953, 1954 and 1955; and at Wooster in 1954 shows that *the less borers the less yield, or the more borers the greater yield.* This parallelism by no means signifies any cause–effect relationship. The difference in borer population in the fall was due to the combined effect of the factor of unattractiveness to egg deposition due to plant-growth condition and the size of the second-brood egg population. The difference in yield in these particular plots, on the other hand, was purely a result of weather conditions which affected the growth of the plants. This contention is supported by the fact that whatever difference is shown in the naturally infested plot (treatment 2), the same difference is shown in the plots where borer infestation of both broods was nil or negligible (treatment 1). (Everett *et al.* 1958, figures 21, 22, and 23.)

In summary, the data presented in this paper have focused attention on the deficiency of the method of estimating crop loss, caused by borer infestation, on the basis of the size alone of the fall population. The present discussion of the population dynamics of the first- and second-brood borers reveals that the fall population may be used as the basis for estimating the yield loss owing to borer damage only when the percentage of summer pupation is zero or very low. The reliability of the estimates based on fall populations becomes progressively less in fields with progressively larger amounts of summer pupa-

tion, and in areas where fields of progressively more varied dates of planting are present. Thus while the fall population may be a reliable basis for estimating the borer population entering hibernation, it is suggested that the summer population is probably a more realistic index for estimating the loss in yield of corn caused by borer infestation in the current year.

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Evaluation of Chemicals for Control of a Spider Mite Complex on Cherry and Peach[1]

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ABSTRACT

Numerous chemicals were evaluated for control of the brown mite (*Bryobia arborea* Morgan and Anderson), the two-spotted spider mite (*Tetranychus telarius* (L.)), and the McDaniel mite (*T. mcdanieli* McGregor) near The Dalles, Oregon during 1955–1959. The entire testing program was conducted in the field using peach (*Prunus persica*) and sour cherry (*P. cerasus*) as the test trees. The materials were applied alone, either as sprays or dusts.

Trithion® (*S*(*p*-chlorophenylthio)methyl) *O,O*-diethyl phosphorodithioate) wettable powder and emulsion (flowable), Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) wettable powder, and Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) wettable powder provided excellent control of all three mite species. Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) wettable powder controlled *T. telarius* and *T. mcdanieli* but not *B. arborea*. Ethion wettable powder was also found to control these two web-spinning species. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) in phosphorothioate), parathion, and malathion wettable powders provided limited and short term control. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl) phosphorodithioate) wettable powder was inconclusive. Sulphenone® (*p*-chlorophenyl phenyl sulfone), ovex, and chlorbenside as wettable powders were all ineffective in controlling the web-spinning group.

Three per cent Tedion dust provided sufficient control on all three species, but must be applied on small populations for maximum results. Three per cent Kelthane dust controlled the brown mite, but not the two-spotted spider mite or the McDaniel mite. Aramite (3%), Tedion (2%), or sulphur (73% or 84%) dust did not control any of the three species.

In the fruit growing district surrounding The Dalles, Oregon, three phytophagous mite species, the brown mite (*Bryobia arborea* Morgan and Anderson), the two-

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spotted spider mite (*Tetranychus telarius* (L.)), and the McDaniel mite (*T. mcdanieli* McGregor), damage the foliage of peach (*Prunus persica*), sweet cherry (*P. avium*), and sour cherry (*P. cerasus*), which are the principal cultivated hosts. Differences in the response of the hosts to these mites are marked and notable differences occur in the mites' biology.

The brown mite, the first to begin feeding in the spring, causes considerable leaf stippling when the population is high. The stippling is not particularly harmful, since infested leaves apparently do not turn brown and drop (Burts 1957), but the feeding does reduce the effective leaf surface. Larvae of the brown mite begin hatching from the overwintering eggs about the time of the green-tip stage of bud development on both peach and cherry. Succeeding generations continue feeding on the foliage throughout the summer. Winter eggs are deposited in the crevices of the bark, on the underside of twigs, small branches, and fruit spurs as early as June or as late as August and September. During June hot, dry weather may reduce the adult population, and the remaining adults will begin ovipositing winter eggs; in cooler summers most of the winter eggs are deposited during August and September (Burts 1957).

Tetranychus telarius and *T. mcdanieli* start feeding later in the season, causing considerable stippling of both cherry and peach leaves. Large populations of the McDaniel mite not only reduce the effective leaf surface but also in hot, dry summers cause the cherry leaves to turn brown and drop (Nielsen 1958). In The Dalles, cherry trees often become partially or completely defoliated by mid-August, resulting in some bloom of sweet cherry in late September. The defoliation of peach is limited, though the leaves are stippled and matted with webbing.

In the spring the overwintering females of the two-spotted spider mite and the McDaniel mite emerge from their winter quarters in the ground or from within bark crevices and begin feeding on wild hosts or foliage on the lower branches of the host trees. Few adults are observed on the foliage during May and most of June. Populations begin building up in the first part of July and in some years a high percentage of the leaves is infested by late July. The average maximum temperature at The Dalles for July and August ranges from 81° to 95° F. Such temperatures apparently are optimum for the McDanie mite; the greatest damage occurs at these temperatures (Nielsen 1958). Also, the largest number of the two-spotted spider mites is found on the foliage during late July and August. In 1922, de Ong found the incubation period of two-spotted spider mite eggs at 87° F. was only one-half as long (3 days) as at 77° F.

In The Dalles dusting is the only practical method of applying insecticides because of a water shortage. With the strong possibility of an adequate water supply in the near future, sprays rather than dusts will be used for insect and mite control because of the increased efficiency obtained with sprays. For this reason a spray program involving the testing of a considerable number of chemicals was begun in 1955. Also, dusting programs were included for comparison with the sprays.

EXPERIMENTAL PROCEDURE.—Each year the mite populations were permitted to reach high populations before chemicals were applied. Testing included spraying or dusting 2- to 8-year-old Montmorency cherry trees and spraying 2- to 3-year-old Improved Elberta peach trees. Dilute sprays were applied to the drip stage, using a 100-gallon portable sprayer and hand gun at 400 p.s.i. Single tree plots were randomized and replicated six times for each treatment. When untreated checks were used, they were included in this plot design. In 1955 and 1956 untreated checks were utilized for comparison with the various treatments and to follow the population trends during late July, August, and early September. Untreated checks were not included in the 1958 and 1959 spray trials (tables 3 and 4), but rather the chemicals were compared one against the other, and with their respective pretest measurements. Untreated checks were used in the 1956 and 1959 dust trials (table 2). Dusts were applied with a commerical ground duster. In 1956 the dusts were applied to single-tree plots randomized and replicated six times, but in 1959 unreplicated, one-half acre plots were used. Six trees in the center of each treatment were selected and sampled. Whether the trials were sprays or dusts, pretest counts were made 1 to 2 days before treatment, the chemicals were then applied and evaluations made at intervals of 1, 3, and 5 weeks following treatment. Measurements ceased at 5 weeks, because the adult brown mite population was usually subsiding naturally and the two-spotted spider mite and the McDaniel mite were migrating to their winter quarters (mid-September).

The following technique was used and found to be a rapid and effective method of screening numerous chemicals for mite control: Twelve leaves per tree were picked from the interior foliage 3 to 6 feet above the ground. Both sides of the 12 leaves from each replication were examined in the field. No attempt was made to count the number of mites per leaf, but the leaves were tabulated as infested or not infested with a given species or group of mites. The infestation on each leaf may have consisted of only a single mite or of numerous mites. The number of infested leaves from the six replications was totaled and divided by 72, giving the percentage of leaves infested for each treatment. The brown mite was tabulated separately from the other two mite species, which were designated as a web-spinning complex (see tables).

The chemicals included in the tests were used as wettable powders, emulsions, and as dusts. The chemicals tested were: malathion, parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), ethion, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), Chlorbenside® (*p*-chlorobenzyl *p*-chlorophenyl sulfide), ovex, Sulphenone® (*p*-chlorophenyl phenyl sulfone), Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical, and Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol).

RESULTS OF SPRAYS ON BROWN MITE, 1956.—Five materials: Kelthane, ovex, Aramite, Trithion, and malathion as wettable powders were tested as postharvest sprays on a small population of brown mite occurring on Montmorency cherry (table 1). Trithion and a "Standard" Kelthane wettable powder provided excellent control, whereas malathion and ovex held the population to

Table 1.—Effectiveness of cover sprays for control of brown mites and web-spinning spider mites on Montmorency cherry, July 28, 1956.

Acaracides	Pounds per 100 Gal.	Per Cent of Leaves Infested							
		Pretest		Post-treatment					
				1 Week		3 Weeks		5 Weeks	
		Brown[a]	Web.[b]	Brown	Web.	Brown	Web.	Brown	Web.
Kelthane 18.5%, WP	2	9.7	41.6	0.0	8.3	0.0	5.5	0.0	4.1
Ovex 50%, WP	0.5	4.1	34.7	1.3	38.8	8.3	56.9	4.1	45.6
Aramite 15%, WP	1.5	15.2	29.1	1.3	2.7	30.5	8.3	29.1	6.9
Trithion 25%, WP	1	1.3	50.0	0.0	0.0	0.0	0.0	0.0	1.3
Malathion 25%, WP	2	1.3	52.7	0.0	18.0	2.7	52.7	1.3	52.7
Untreated	—	8.3	36.1	6.9	44.4	12.5	80.5	12.5	62.5

[a] *Bryobia arborea.*
[b] *Tetranychus telarius* and *T. mcdanieli.*

a low level. In 3 weeks the percentage of infested leaves in the Aramite plots increased to double that of the pretest measurement.

Results of Dusts on Brown Mite, 1956 and 1959.—Dust formulations of Kelthane 3%, Aramite 3%, Tedion 2% and 3%, dusting sulphur 84% and dusting sulphur 73% in combination with 10% lead arsenate were tested on Montmorency cherry at the rate of 55 pounds per acre (table 2). These dusts were applied following cherry harvest.

In 1956, 73% dusting sulphur in combination with lead arsenate, applied when 9.7% of the leaves were infested, provided excellent control. When 84% dusting sulphur was applied in 1959 to a high leaf infestation (97.2%), results were poor. Considerable leaf scorch was noted in 1959 and repeated applications of dusting sulphur may have resulted in additional injury.

With the use of Aramite in 1956 and 1959 the percentage of leaves infested in the treated plots was higher than in the checks at the end of 5 weeks. Tedion dusts, 2% and 3%, were tested during 1959. The 3% material gave better initial kill 1 week following application, but at the 3-week period, results with the 2% and 3% dusts were equal, even though 3% provided better control in 5 weeks. Though two distinct levels of infestation were present in 1956 and 1959, Kelthane dust gave excellent control for a period of 5 weeks in both tests.

Results of Sprays on the Web-Spinning Complex, 1955–1959.—The chemicals tested on Montmorency cherry for control of *T. telarius* and *T. mcdanieli* were, with the exception of Trithion emulsion (flowable), all wettable powders (tables 1 and 3). All were applied as postharvest sprays.

Malathion provided limited control for approximately 3 weeks when applied to a low infestation in 1955 (table 3). In 1956, when 52.7% of the leaves were infested, the percentage of infestation 3 weeks after application was at the pretest level (table 1). Parathion was tested for only 1 year (1959) and after 5 weeks the infestation was above the pretest measurement (table 3).

Other organic phosphates included in the tests were Diazinon, ethion, Guthion, Trithion WP and Trithion E (flowable). Diazinon provided excellent control in 1958, but control in 1959 was comparable to parathion (table 3). In 1958, ethion gave 100% control within 1 week, but a low infestation remained in 1959 (table 3). At the rate

Table 2.—Effectiveness of dusts[a] for control of brown and web-spinning spider mites on Montmorency cherry foliage.

Acaracides	Per Cent of Leaves Infested							
	Pretest		Post-treatment					
			1 Week		3 Weeks		5 Weeks	
	Brown	Web.	Brown	Web.	Brown	Web.	Brown	Web.
July 28, 1956								
Kelthane, 3%	12.5	40.2	0.0	5.5	4.1	16.6	2.7	24.7
Aramite, 3%	6.9	26.3	0.0	11.1	11.1	16.6	20.8	16.6
Lead arsenate sulphur, 10%–73%	9.7	36.1	0.0	26.3	2.7	40.2	1.3	54.1
Untreated	8.3	36.1	6.9	44.4	12.5	80.5	12.5	62.5
July 29, 1959								
Kelthane, 3%	91.6	37.6	4.1	15.2	4.1	29.1	0.0	29.1
Aramite, 3%	100.0	50.0	91.6	66.6	73.6	86.1	54.1	80.5
Sulphur, 84%	97.2	44.4	29.1	31.1	37.6	29.1	13.8	56.9
Tedion, 2%	94.4	63.8	56.9	56.9	11.1	34.7	12.5	31.9
3%	90.2	62.5	24.7	23.6	11.1	8.3	1.3	9.7
Untreated	94.4	63.8	95.8	83.3	77.7	87.5	41.6	88.8

[a] Applied at 55 pounds per acre.

Table 3.—Effectiveness of cover sprays for control of a web-spinning spider mite complex on Montmorency cherry.

Acaracides	Pounds per 100 Gal.	Per Cent of Leaves Infested			
		Pre-test	Post-treatment		
			1 week	3 weeks	5 weeks
August 12, 1955					
Kelthane 18.5%, WP	2	18.0	8.3	20.8	37.6
Chlorbenside 20%, WP	1	15.2	13.8	59.7	80.5
Ovex 50%, WP	0.5	5.5	9.9	13.8	61.1
Aramite 15%, WP	1.5	8.3	1.3	2.7	37.6
Malathion 25%, WP	2	13.8	2.7	29.1	56.9
Untreated	—	15.2	24.7	54.1	77.7
August 18, 1958					
Kelthane 18.5%, WP	1.5	82.0	1.3	0.0	0.0
Tedion 25%, WP	1	72.2	2.7	0.0	0.0
Sulphenone 50%, WP	1.5	66.6	40.2	34.7	33.0
Trithion 25%, WP	1	79.1	0.0	0.0	0.0
Trithion (flow.) E, 43.7%	0.5[a]	83.3	0.0	0.0	0.0
Diazinon 25%, WP	2	88.8	0.0	0.0	0.0
Ethion 25%, WP	1	86.1	0.0	0.0	0.0
Guthion 25%, WP	3	80.5	0.0	0.0	0.0
August 11, 1959					
Kelthane 18.5%, WP	1.5	52.7	0.0	1.3	1.3
Tedion 25%, WP	1	44.4	11.1	5.5	6.9
Aramite 15%, WP	1.5	55.5	1.3	6.9	18.0
Trithion 25%, WP	1	37.6	0.0	0.0	1.3
Trithion (flow.) E, 47.3%	0.5[a]	29.1	0.0	0.0	0.0
Diazinon 25%, WP	2	43.0	4.1	22.2	36.1
Ethion 25%, WP	1	50.0	2.7	8.3	6.9
Guthion 25%, WP	1.5	51.4	6.9	12.5	6.9
Parathion 25%, WP	1	29.1	2.7	15.2	38.8

[a] One-half pint.

of 3 pounds of wettable powder per 100 gallons of water, Guthion provided 100% control in 1 week (table 3). In 1959, the dosage was reduced to 1.5 pounds per 100 gallons of water, but at this dosage the population was building up in 3 weeks (table 3). Trithion wettable powder gave excellent and consistent results in 1956, 1958, and 1959 (tables 1 and 3), while the emulsion gave comparable results for 1958 and 1959 (table 3).

The sulphur-based compounds: Chlorbenside, Sulphenone, ovex, Aramite, and Tedion were also included in the screening program. Chlorbenside and Sulphenone provided virtually no control, reducing the population only slightly for a period of 1 week (table 3). For the two consecutive years (1955 and 1956) that ovex was tested, the percentage of leaves infested was higher than the pretest measurement 1, 3, and 5 weeks following application (tables 1 and 3). In 1955 Aramite, applied to a small initial infestation (8.3%), maintained control for a period of 3 weeks but at the 5-week period the percentage of infestation had built up to 37.6%, while the check attained a 77.7% level (table 3). In 1956 control was maintained for 5 weeks, while the population in the check reached a level of 62.5% leaf infestation (table 1). The third test in 1959 indicated excellent control for 3 weeks, with 18.0% of the leaves infested at 5 weeks, while the pretest count showed 55.5% leaf infestation (table 3). The results with Tedion in 1958 were excellent. Control was attained 100% in 1 week (table 3). In 1959 the material was slow in bringing the population under control. The initial kill 1 week after application was the poorest of all materials tested. Only a small percentage of the leaves was populated with mites during the 3 and 5 weeks following application (table 3).

Two Kelthane wettable powders were tested. A formulation of "Standard" wettable powder (18.5%) at the rate of 2 pounds per 100 gallons of water was tested in 1955 and 1956 (tables 1 and 3). In 1958 and 1959 a Micro-cel wettable powder (18.5%) was used at the rate of 1.5 pounds per 100 gallons of water. The latter formulation provided excellent control within 1 week after application (table 3).

Improved Elberta peach trees were treated in 1958 and 1959, to determine if there was any phytotoxic effects on fruit or foliage resulting from use of the following chemicals; parathion, Diazinon, ethion, Guthion, Trithion, Sulphenone, and Kelthane wettable powders along with Trithion emulsion (flowable). The results tabulated in table 4, show that Trithion wettable powder and emulsion provided consistent and excellent control. The 1959 trial with Kelthane was similar to that obtained on Montmorency cherry.

RESULTS OF DUSTS ON THE WEB-SPINNING COMPLEX, 1956–1959.—The most promising of the chemicals was 3% Tedion (table 2), while dusting sulphur apparently offered little possibility (table 2). Three per cent Kelthane and Aramite were not adequate.

DISCUSSION.—Summer control of the brown mite and subsequent reduction of winter eggs on cherry can be attained by at least two approaches. One method is combining an acaricide with one of the applications made against a cherry fruit fly (*Rhagoletis cingulata indifferens* Curran) in June or the use of a single chemical suitable for both fruit fly and brown mite control. A second is by application of an acaricide in early July, immediately after cherry harvest. Peaches should also be treated during June or early July for control of this mite.

In mid-July an acaricide is also needed on cherry for control of the two-spotted spider mite and the McDaniel mite before the population has become large and damaging. The application could be made in combination with the last fruit fly cover spray or immediately after harvest. Control measures will also need to be applied to peach at this same time.

The presence of the three mite species on peach and cherry presents a problem of selecting an acaricide that will control all three, rather than a material that will control one species and not the others. The possible presence of unharvested fruit (cherry) when applications are made in early July further limits the use of certain chemicals,

Table 4.—Effectiveness of cover sprays for control of a web-spinning spider mite complex on Elberta peach.

Acaracides	Pounds per 100 Gal.	Per Cent of Leaves Infested			
		Pre-test	Post-treatment		
			1 week	3 weeks	5 weeks
August 18, 1958					
Tedion 25%, WP	1	75.0	0.0	0.0	0.0
Sulphenone 50%, WP	1.5	78.3	41.6	34.7	30.0
Trithion 25%, WP	1	70.8	0.0	0.0	0.0
Trithion (flow.) E, 43.7%	0.5[a]	59.7	0.0	0.0	0.0
Diazinon 25%, WP	2	65.2	0.0	0.0	0.0
Ethion 25%, WP	1	82.0	0.0	0.0	0.0
Guthion 25%, WP	3	86.1	0.0	0.0	0.0
August 11, 1959					
Kelthane 18.5%, WP	1.5	63.3	1.6	3.3	3.3
Tedion 25%, WP	1	54.1	36.1	12.5	12.5
Trithion 25%, WP	1	63.3	0.0	1.6	3.3
Trithion (flow.) E, 47.3%	0.5[a]	72.2	0.0	1.3	1.3
Diazinon 25%, WP	2	62.5	9.7	43.0	50.0
Ethion 25%, WP	1	69.4	1.3	5.5	6.9
Guthion 25%, WP	1.5	38.8	6.9	33.2	33.2
Parathion 25%, WP	1	77.7	2.7	44.4	44.4

[a] One-half pint.

because of possible residues. Peach harvest usually occurs in mid-August and September, thus presenting a slightly different residue problem.

The chemicals, as sprays or dusts, were tested under rather severe conditions. Populations were allowed to build up to a high level before the materials were applied. An abundance of webbing and accumulated field dust on the foliage offered protection for the web-spinning mites. Apparently the populations of the two-spotted spider mite and the McDaniel mite were increasing and expanding, since the percentage of infested leaves increased in the check during late July, August, and early September (tables 1, 2 and 3). At The Dalles, high winds and low humidity during daylight hours apparently affect the deposit of chemical dusts on the leaves of cherry. Chemical analysis of cherry leaves treated with insecticide dusts for fruit fly control have indicated less material on the leaf surface (milligrams per square centimeter) than on those treated with the same material in the less windy and more humid Willamette Valley of Oregon.

Aramite appeared to be selective, controlling the web-spinning complex for a period of 3 to 5 weeks, but not the brown mite. Furthermore, Aramite does not have clearance for use on peach and cherry after edible parts have started to form.

Kelthane wettable powder gave excellent results against the three mite species on cherry and equal results on the web-spinning group on peach. This acaricide offers the possibility of use within 7 days of harvest on cherries and 14 days on peaches, provided there has not been prior usage within 30 days. Three per cent dust was adequate for control of brown mite, but did not control the web-spinning complex. Possibly a higher percentage of ingredient would provide control. This possibility needs further investigation.

The use of organic phosphates as insecticides or acaricides is limited in The Dalles area, consequently resistance to organic phosphates is not currently widespread. Trithion wettable powder or emulsion (flowable) has provided control of the web-spinning complex on peach and cherry. A 1-year test (1956) of Trithion wettable powder on a small population of brown mite indicated its promise for control of this mite, but it has the limitation that it cannot be applied within 30 days of harvest. As an acaricide it has excellent promise as a postharvest spray on cherries and on the late maturing peach varieties Improved Elberta and J. H. Hale. These varieties are usually harvested from mid- to late August and early September.

Parathion controlled the web-spinning complex for a period not exceeding 2 weeks. Malathion was not satisfactory for control of a large population of the two-spotted spider mite and the McDaniel mite even though a 1-year test indicated it would control a small population of brown mite. The erratic results with Diazinon over a period of 2 consecutive years against the web-spinning group cannot be readily explained. Experimentally in 1958 and 1959 ethion provided consistent results in control of the web-spinning complex. Possibly the dosage of ethion is too low and should be increased to 1.5 pounds per 100 gallons of water instead of the 1 pound as tested. Further data are needed on the use of Guthion on peach and cherry.

Tedion wettable powder and 3% dust showed promising results for control of all three mites. In all probability, owing to the slowness of the initial kill, the material will give better results if applied when the populations are small, rather than when they are large, as they were when the tests were conducted.

Dusting sulphur was the only material that injured the foliage of Montmorency cherry. The chemicals tested on Improved Elberta peach did not injure the fruits or foliage (table 4).

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Problems in the Control of Mint Insects[1]

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ABSTRACT

Of the 54 species of insects and related pests found on peppermint and spearmint in Indiana, only cutworms and the mint looper, *Raphoplusia ou* (Gn.), have been serious problems in the past 10 years. The mint flea beetle, *Longitarsus waterhousei* Kut., has not been abundant since growers adopted a 2-year crop rotation for mint. Several species of leafhoppers and plant bugs have been present every year, but losses from their feeding have been slight.

Cutworms were present in mint fields both early in the season and again near harvest. Chlordane, aldrin, dieldrin, and heptachlor gave good control of these pests when applied in late April and early May as the first new growth was appearing. Cutworms present in July and August were controlled by a single application after the crop was cut.

The mint looper was found to have two generations a year

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with the peaks of larval abundance in late June and again in mid-August. Of numerous insecticides tested against the looper, dieldrin, heptachlor, and DDT were the best. Two applications at a 10-day interval in late June gave excellent control. Malathion was found to be of value against spider mites and to some extent against plant bugs and leafhoppers.

Of the materials used as foliar sprays only malathion has had a tolerance established and usage labels approved. Apparent residues found in mint oil from heptachlor- and dieldrin-treated plots were quite variable in different years and in some instances were as high as 2.7 and 4.7 p.p.m., respectively. Methods of analyzing for residues in mint oil, especially for dieldrin, toxaphene, and DDT, have not been satisfactory. DDT, however, has been the standard treatment for mint insects since 1947.

The use of insecticides on mint foliage created another problem—that of possible off-flavor in the oil. Oil samples from treated plots were sent to company experts for flavor evaluation and the following materials were approved for use: DDT, dieldrin, chlordane, heptachlor, toxaphene, Strobane® (a terpene polychlorinate with a chlorine content of approximately 66%), malathion, parathion, EPN-300, and Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O*,*O*-dimethyl phosphorothioate). Two materials, aldrin and lindane, were listed as questionable, whereas BHC was objectionable.

The peppermint and spearmint crops in Indiana are attacked by more than 50 insect pests, although only a few are of economic importance. The mint looper, *Raphoplusia ou* (Gn.), has been the most serious problem on mint and has caused extensive losses about 1 year out of 4. The mint flea beetle, *Longitarsus waterhousei* Kutschera, first observed in the State in 1926, has not been a serious problem since the growers changed to a 2-year rotation for mint. Other pests, such as cutworms, leafhoppers, plant bugs and grasshoppers, are frequently present in mint fields. During the past 10 years, extensive tests have been made in Indiana to determine the value of various insecticides in the control of the pests of peppermint and spearmint.

Peppermint and spearmint are specialty crops grown primarily in two regions: the Midwest muck area in Indiana and Michigan and the West Coast area in Washington and Oregon. The average acreage of these two crops in Indiana in recent years has ranged between 14,000 and 24,000 acres, with an average oil yield of 33 pounds per acre. The big reduction in acreage and the increase in acre yield during the past 30 years can be attributed in part to the discontinuance of small fields by the general farmer and the concentration of mint acreage on specialized farms. While acreages and yields are both higher on the West Coast, the Midwestern farmers have stayed with mint production because of lower costs and the preference of the larger oil users for the quality of the Midwestern oils.

Insect abundance has often been a factor in the economical production of mint oil. More than 54 species of insects and related pests have been found associated with peppermint, spearmint and wild mint in Indiana and neighboring states. These are as follows:

COLEOPTERA

Longitarsus waterhousei Kutschera, mint flea beetle
L. suspectus Blatchley
Chaetocnema denticulata (Ill.), toothed flea beetle
C. pulicaria Melsh., corn flea beetle
Systena blanda Melsh., pale-striped flea beetle
S. frontalis Fabr.
Phyllotreta zimmermanni Cr.
Psylliodes punctulata Melsh., hop flea beetle
Epitrix fuscula Crotch, eggplant flea beetle
E. cucumeris (Harr.), potato flea beetle
Pachybrachys atomarius Melsh., on wild mint only
P. othonus Say, on wild mint only
Zygogramma suturalis casta Rogers
Lema trilineata (Oliv.), three-lined potato beetle
Phyllophaga spp., white grubs (root injury by larvae)
Macrodactylus subspinosus (F.), rose chafer
Melonotus spp., wireworms (root injury by larvae)
Dalopius sp., wireworm (root injury by larvae in Wisconsin)

DIPTERA

Leafminers, species unknown
Tipula mingwe Alex., leather jacket (from Michigan)

HOMOPTERA

Philaenus leucophthalmus (L.), meadow spittlebug
Empoasca fabae (Harris), potato leafhopper
Macrosteles fascifrons (Stål), six-spotted leafhopper
Norvellina seminuda (Say)
Nasutoideus frontalis (V. D.)
Porodon menthae (Buckton), mint aphid

HEMIPTERA

Mormidea lugens (Fabr.)
Euschistus variolarius (P. de B.) one-spot stink bug
Nysius ericae (Schilling), false chinch bug
Corizus lateralis (Say)
Lygus lineolaris (P. de B.), tarnished plant bug
Adelphocoris rapidus (Say), rapid plant bug
Trigonotylus ruficornis (Goef.)
Neurocolpus nubilus (Say)

LEPIDOPTERA

Raphoplusia ou (Gn.), mint looper
Heliothis zea (Boddie), corn earworm
Papaipema nebris (Guen.), stalk borer
Pyrausta nubilalis (Hbn.), European corn borer
Peridroma margaritosa (Haw.), variegated cutworm
Agrotis ypsilon (Rott.), black cutworm
A climbing cutworm, not identified
Sparganothis sulfureana Clemans
Isia isabella (J. E. Smith), banded woollybear
Ceramica picta (Harris), zebra caterpillar
Loxostege similalis (Guen.), garden webworm
A leaf tier, not identified

ORTHOPTERA

Melanoplus differentialis (Thomas), differential grasshopper
M. femur-rubrum (DeG.), red-legged grasshopper
Long-horned grasshoppers, possibly several species

THYSANOPTERA

Thrips tabaci Lind., onion thrips
T. nigropilosus Uzel, chrysanthemum thrips
Frankliniella gilmorei Morgan (described from mint in Tennessee)

OTHER ARTHROPODS

Tetranychus telarius (L.), two-spotted spider mite
A millipede, not identified

MINT LOOPER.—Of these pests, the mint looper (fig. 1) has been the most destructive. It has been present every year and in some seasons, such as 1949 and 1957, was

FIG. 1.—Mint loopers on peppermint foliage.

sufficiently abundant to cause serious losses in untreated fields. The insect had two generations a year, with the larvae of the first brood appearing around June 20 and those of the second about August 10. When the populations were high, the first-brood larvae, feeding from mid-June to mid-July, removed most of the oil-producing leaves. Reductions in yield were high unless the grower delayed harvest from the normal August 1 period to allow the plants to grow more leaves. Losses in the severe infestation of 1957 ranged from 25% to 75% of the normal yield. The second brood in August was considered of little importance unless the grower planned to cut his crop a second time. A virus disease and several parasites were important factors in holding looper populations to noneconomic levels in most years.

FLEA BEETLE.—The mint flea beetle can still be found in most mint-producing areas of Indiana, but losses in the past 15 years have been negligible. The change in rotation with 2 years in mint, made to reduce losses from certain plant diseases, has prevented the flea beetle from becoming established. During these studies, cutworms of several species frequently damaged newly set mint or the new growth on meadow mint in the spring. A subterranean species of cutworm, the presence of which was often unsuspected by the grower, caused much of this loss. Other pests frequently found abundant in restricted areas included several species of plant bugs, leafhoppers, and grasshoppers. In dry summers or in the drier border areas of fields, the two-spotted spider mite frequently became a problem in July and August.

Calcium arsenate, lead arsenate, paris green, and cryolite were recommended for flea beetle control before the change in crop rotation and the introduction of the newer insecticides occurred. The first observation on the effectiveness of DDT against leafhoppers and other pests were made on the Purdue Muck Crop Farm in 1946. Potatoes were being sprayed in an adjacent field. In order to run out the material in the spray tank, the four outside rows of peppermint were treated on four dates. This field had a heavy population of leafhoppers, plant bugs, and some loopers. The value of the treatment was apparent at cutting time when the plants in the treated rows were a foot taller and the oil yield was proportionately higher.

EXPERIMENTATION.—Experimentation with the mint crop has presented many problems, as uniform stands and growth in the field have been difficult to obtain. Although the crop is propagated by root cuttings, or occasionally by transplants, uniform stands are rare. Even on second-year meadow mint, stand and growth vary considerably. In experimental work, the plot size has been varied from 1/20-acre with 12 replications to 1-acre plots with four replications. Differences in yield have seldom been sufficient to show that one treatment was significantly better than another, or even better than the untreated. Since an important criterion in evaluating treatments was the quality of the oil, plot size was eventually set at one-half acre or more, so that commercial tubs could be used in distillation.

1949 Tests.—The first tests, in 1949, were made at the Purdue Muck Farm and on a neighboring farm. Populations of the mint looper were high and some growers made two applications of DDT by airplane. In the trials at the Purdue Farm, 24 materials or mixtures were applied on 5 dates from June 20 to July 26. No foliage injury from any of the materials was observed. Plots were cut 15 to 18 days after the last treatment and were distilled in four small stills The weight of the green hay cut showed a high degree of correlation with the weight of oil produced. Some adjustments had to be made in yields to compensate for missing plants and death of plants from verticillium wilt. Table 1 lists the materials used with the exception of three, which have since been dropped from commerical production.

Insect populations in the various plots were determined by making net-sweeps on July 14 and 25, and August 10. Five groups of insects were counted: plant bugs, potato leafhopper, six-spotted leafhopper, meadow spittlebug adults, and grasshopper adults and nymphs. The six-spotted leafhopper was the most common of the five groups with an average of 38 per 20 sweeps on all plots on the first date, 29 on the second date, and 152 on the third. Figures for the others were as follows: spittlebug adults 25, 28, 123; plant bugs 2, 3, 19; potato leafhopper 5, 7, 40; and grasshoppers 3, 3, 16. Two materials, BHC and parathion, gave a rapid kill, as indicated by the fact that most insects were on the ground, dead or dying, in less than 24 hours.

On the farm of the cooperator, five materials or mixtures of materials were applied on three dates. Materials tested included DDT, TDE, parathion and a fungicide,

Table 1.—Yield of oil from peppermint plots receiving five applications of various insecticides, 1949.

TREATMENTS	ACTUAL INSECTICIDE APPLIED PER ACRE (LB.)	OIL YIELD PER PLOT (ML.)
Chlordane WP	5.0	101.1
DDT WP	5	83.7
BHC WP (12% gamma)	1.25	82.0
TDE EC	5	79.8
BHC WP (50% gamma)	1.25	79.3
Aldrin WP	2.5	78.9
Chlordane EC	5	78.8
Dieldrin EC	2.5	78.3
Toxaphene EC	5	74.2
DFDT[a] EC	5	74.2
Methoxychlor	5	73.5
DDT colloidal	5	72.4
Prolan®[b]	2.5	71.2
DFDT WP	5	71.1
Untreated	—	68.9
Parathion WP	1.25	67.0
TDE WP	5	65.5
Dieldrin WP	2.5	64.4
Aldrin EC	2.5	57.7
Toxaphene WP	5	50.1
DDT EC	5	45.7
Methoxychlor EC	5	44.9
L.S.D. 19:1	—	7.4
99:1	—	n.s.

[a] 1,1,1-trichloro-2,2-bis(*p*-flurophenyl)ethane.
[b] 1,1-bis(*p*-chlorophenyl)-2-nitropropane.

zineb. The highest yield was on the DDT plots with an average of 72 pounds of oil per acre as compared with 66 pounds on the untreated. Other yields were similar but no significant differences between treatments occurred.

In 1949, cutworms caused extensive damage to newly planted row mint and to young meadow mint in early May. Several growers sprayed their fields with chlordane and obtained excellent control. With the exception of spittlebugs, other insects were present in normal numbers. Spittlebug populations were high in many fields in July and August, when the adults migrated in from adjacent legume and pasture areas. Normally this insect was unable to develop on mint stems because fall plowing buried the plants too deep for the young to emerge. Only once during this study were nymphs and their spittle masses observed on mint. This was in a small area on the Purdue Farm missed in the fall plowing.

1950 Tests.—In 1950, tests at the Purdue Farm included 10 materials applied on 5 dates to plots replicated 6 times. Materials included DDT as a wettable powder and as an emulsifiable concentrate, parathion wettable powder, EPN-300, lindane, dieldrin, chlordane, toxaphene, Dilan (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) and experimental acaricide (C1006). Of these materials, only Dilan caused injury and this injury was insufficient to reduce the yield. Since the insect populations were low and considerable variation occurred among replications of the same treatment, the differences in yield between the treatments were not significant.

1951 Tests.—Three insecticides, DDT, aldrin, and EPN-300, were tested in 1951. Materials were applied to peppermint on five dates between June 19 and July 25. Population counts of four groups of insects were made just before, and 24 hours after, the fourth application on July 16. These figures, along with the yield of oil (table 2), show that the DDT-treatment gave the highest yield and also the best kill of the insects.

1952 Tests.—Four insecticides were tested in 1952 on peppermint. Because three of the four replications had to be set with plants, the crop was so late that only six applications of the insecticides were made between June 24 and July 30 The average yields of oil from the different plots were as follows:

NPD (a phosphate compound)	1,131 ml.
DDT	1,050 ml.
Heptachlor	976 ml.
Aldrin	948 ml.
Untreated	822 ml.
L.S.D. 19:1	110
99:1	n.s.

1953 Tests.—In 1953, four materials, DDT, Strobane® (a terpene polychlorinate with a chlorine content of approximately 66%), toxaphene, and malathion, were tested against the insects present on peppermint. Both cutworms and loopers were more abundant than usual, while leafhoppers and plant bugs were present in normal numbers. Cutworms were especially abundant on July 24 when a population count showed an average of seven per square foot under the untreated plants. At harvest, differences in yields between the treatments were not significant.

1954 Tests.—During the 1954 season, loopers and cutworms were again the major insect problems. In order to obtain oil samples for residue analysis and flavor evaluation, five materials were applied to a single plot each on three dates, June 15 and 30 and July 12. The hay from each plot was distilled through one tub and samples of the oil were sent to two cooperators for chemical analysis and flavor evaluation. The analysis of insecticide residues in mint oil have always presented problems, especially for dieldrin. One company reported the following residues found in the oil:

Heptachlor 3.0 p.p.m. (1½ pounds actual applied)
Dieldrin 1.0 p.p.m. (1 pound actual applied)
DDT 9.8 p.p.m. (4½ pounds actual applied)
Strobane® 8.4 p.p.m. (6 pounds applied)
Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate) 34.4 p.p.m. (½ pounds actual applied)
Untreated 0.2 p.p.m.

Another company reported the residue in oil from the heptachlor-treated plot as only 0.54 p.p.m.

1955 Tests.—In 1955, a cooperative test involving growers in Michigan, Wisconsin, and Indiana was arranged to evaluate the use of insecticides under uniform conditions. The land used in the four locations involved in the cooperative test had never had a soil treatment nor had a treatment been applied to a crop growing on it. A second requirement was that foliar applications be made about 10 days before harvest, with no rainfall during that period. One treatment in each location was a broadcast application of dieldrin at 5 pounds actual per acre disked into the soil before planting in the spring. For foliar applications, dieldrin was applied at 6 ounces actual and malathion at 1 pound actual per acre. In Indiana, one of the fields used was planted to peppermint and the other to spearmint.

Residues in the oil samples were analyzed by chemists

Table 2.—The yield of oil from peppermint treated five times with various insecticides and the number of certain insects caught in 100 sweeps made before and 24 hours after the fourth application on July 16, 1951.

Treatments	Pounds Actual Insecticide per Acre	Yield of Oil (ml.)	No. Spittlebug Adults		No. Insects Caught per 100 Sweeps					
					Empoasca		Other Leafhoppers		Plant Bugs	
			Before	After	Before	After	Before	After	Before	After
DDT EC	5.0	1,400	395	22	7	2	43	2	6	2
Aldrin EC	3.7	1,090	389	100	18	8	54	38	9	0
EPN-300 EC	3.0	815	485	270	11	2	52	12	23	0
Untreated	—	906	427	384	14	18	33	120	6	10
L.S.D. 19:1		456								
99:1		n.s.								

of the Beech-Nut Company. Their results showed that spearmint oil grown in Indiana on dieldrin-treated soil had an apparent residue of 4.3 p.p.m. Where the foliage was sprayed with dieldrin, the residues were the same on spearmint and on peppermint, 0.8 p.p.m. Plots sprayed with malathion had no residues in the oil from either peppermint or spearmint. A possible explanation of the large amount of residue found in the dieldrin-treated Indiana soil was the soil type and method of harvest. The soil was a loose, sand and muck mixture, which at harvest was very dry. As the dry hay was gathered up by the chopper, much loose dirt went into the wagons and eventually into the tubs for distillation. It is possible that much of the residue came from the soil rather than the foliage, as the soil treatment was applied before roots were planted some 90 days before harvest.

An additional test was run on the Purdue Farm where heptachlor was used at a rate of 12 ounces actual per acre in one application on July 20. The mint was cut 10 days later and on analysis the residue found was 2.43 p.p.m.

1957 Observations.—The mint looper population in 1957 was the highest in the past 25 years. In fields all across northern Indiana growers estimated losses at 50% to 75% of the expected yield where no treatments were applied. The first loopers were observed June 20 and by July 18 most caterpillars had pupated. Defoliation during this period was serious, but by early August plants again had a heavy set of leaves. A second brood of larvae developed after the middle of August, but was of little concern to growers, as little mint was harvested a second time.

The three principal materials used in fighting this outbreak and in our experimental trials were DDT, dieldrin, and heptachlor. All three gave satisfactory control, although dieldrin gave outstanding results. Oil from DDT-treated areas was sent to two companies for an analysis of the residue present, but both reported that no method was known that would detect small quantities of DDT in the oil. One application of heptachlor applied at a rate of 8 ounces actual per acre a month before harvest left residues of 0.2 p.p.m. according to Purdue chemists and 0.4 p.p.m. according to chemists of the manufacturer. No reports were received on residues present in samples of oil made from dieldrin-treated plants sent to two companies.

1958 Tests.—In 1958, a single application of aldrin, dieldrin, and heptachlor in granulated formulations was applied to peppermint plots. Insects were not abundant. All materials were applied at a rate of 1 pound actual per acre on August 19 and many of the granules adhered to the foliage. Harvest was September 5. Samples of oil from each of the plots were tested by chemists of the Beech-Nut Life Savers Corporation and the following quantities of insecticides were detected by the total-chlorine method:

Untreated control	0.00 p.p.m.
Heptachlor	2.70 p.p.m.
Dieldrin	2.80 p.p.m.
Aldrin	2.40 p.p.m.

1959 Tests.—A commercially produced microbial insecticide made from spores of *Bacillus thuringiensis* Berliner was tested in 1959 against the mint looper on three farms. It was used as a spray on areas in large commercial fields at rates of 2, 3, and 4 pounds of the formulation per acre. On the first field, the material was applied June 20 in anticipation of a looper population that never developed. A second field was treated early in July and again the population was too low to give any information on control. A third field on the Purdue Farm received an application of 2 pounds on July 11. Six days later the peppermint plants were examined and a heavy infestation of young loopers was found in the treated as well as in the untreated areas. No reduction in numbers from the treatment was apparent.

Off-Flavor.—The use of insecticides has, in some instances, produced another serious problem, that of off-flavor in the mint oil. Peppermint and spearmint oils, like other essential oils, are subjected to rigid standards of quality. The flavor of the oil may be affected by numerous conditions, such as the presence of weeds in the harvested crop, the stage of growth at cutting, and the presence of certain insecticides, fungicides, and herbicides on the foliage. Each company using any considerable quantity of mint oil has a panel of specialists to determine if the flavor and odor meet their specifications. Commercial peppermint and spearmint oils are blends from many fields that have been mixed to satisfy the specialists. The specialists from several companies have cooperated with us and have judged the quality of oils from insecticide-treated plots. While a sample from a single plot or a composite sample from the replications of a single treatment could not be compared with the standard Indiana oil, the specialists did compare samples from a treatment with a sample from untreated plants in the same field. Considerable disagreement occurred in the ratings of oil samples, but over a 10-year period the specialists have agreed that the following materials used on mint foliage 10 to 14 days before cutting caused no off-flavor in the oil: DDT, di-

eldrin, heptachlor, chlordane, toxaphene, Strobane, malathion, parathion, EPN-300, and Chlorthion. Aldrin and lindane were listed as questionable, whereas BHC was objectionable.

DISCUSSION.—Of the numerous insects found on peppermint and spearmint, the mint looper was the most destructive. Cutworms were frequently abundant early in the season and caused damage to new growth of row and meadow mint. Leafhoppers and plant bugs were common on mint and undoubtedly had some influence on plant growth. Of the insecticides tested, DDT was the most satisfactory, as it gave excellent control of leafhoppers, plant bugs, and adult spittlebugs, and good control of loopers and other caterpillars. Dieldrin gave excellent control of loopers, cutworms and caterpillars, while heptachlor, Strobane®, and toxaphene were also satisfactory against the same pests.

From these tests, and from others in Michigan, Oregon, and Washington, data have been submitted and a tolerance established for the use of malathion on mint foliage. The following usages have been accepted by the Pesticides Regulation Branch of the U. S. Department of Agriculture:

1. Aldrin up to 5 pounds actual toxicant per acre in the soil in late April or in May for soil insect control.
2. Dieldrin up to 5 pounds actual used as in 1.
3. Heptachlor[3] up to 7½ pounds actual used as in 1.
4. Dieldrin up to 5 pounds actual applied on soil surface in late April or early May, but not disked into the soil for cutworm control.
5. Heptachlor[3] at 8 to 12 ounces actual applied on soil surface or over the row in late April or early May for cutworm control.
6. DDT at 3 pound actual per acre applied to foliage as needed. DDT was recommended on mint as early as 1948 (Gould 1948) but the continued acceptances of this use is being carefully re-evaluated.

Applications of dieldrin, heptachlor, and toxaphene to the foliage of mint has not been approved, as these materials present a more complex problem. Analytical methods for detecting them in mint oil are at present inadequate or, where a method is available, the apparent residues found have been high. A recent change in the thinking of the Federal officials indicates that new residue data on the green mint hay must be submitted.

REFERENCE CITED

Gould, G. E. 1948. Insect pests of muck crops. Purdue Univ. Agric. Expt. Sta. Circ. **338.**

[3] A zero tolerance of heptachlor and heptachlor epoxide was published in the Federal Register of January 19, 1960. Consequently the acceptances of heptachlor usage on mint will probably be withdrawn.

Control of the Grape Leaf Folder in California[1]

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ABSTRACT

Results are given of field tests made in 1958 and 1959 to control the larvae of the grape leaf folder, *Desmia funeralis* (Hbn.). Sprays of endrin, Sevin® (1-naphthyl-N-methyl carbamate), acid lead arsenate, and spores of *Bacillus thuringiensis* (Berliner) were effective in controlling first-brood larvae. Control was less persistent with *Bacillus* spores. Dusts of endrin, Sevin, and parathion were effective in controlling second-brood and third-brood larvae, but the *Bacillus* dust used was not effective. Sevin appeared to be compatible with Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) as a combination dust. Of previously untried insecticides, dimethoate and Bayer 22408 (*O,O*-diethyl *O*-naphtalimidophosphorothioate) were very promising, while Kepone® (-decachloroöctahydro-1,3,4-methano-2*H*-cyclobuta[cd]pentalen-2-one) and Methyl-Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-dimethyl phosphorodithioate) were not satisfactory for control of third-brood larvae. The effectiveness of the insecticides tested on both grape leaf folders and grape leafhoppers, *Erythroneura elegantula* Osb., is discussed.

The grape leaf folder, *Desmia funeralis* (Hbn.), has been a problem of increasing importance in the San Joaquin Valley since 1954. The common name of this insect was derived from the habit in the Eastern United States of a strain which folds leaves and feeds within this protection. The California strain makes pencil-size rolls and feeds on the inner margins of the rolls. In California the insects are commonly called leafrollers and the larvae are called roller-worms. Damage consists of defoliation with consequent reduction of fruit quality and weakening of the vines. In late season the larvae may also attack the fruit. American grape varieties are preferred. Other plant hosts recorded are evening primrose, Virginia creeper, and red bud.

There are three full broods and often a more-or-less-complete fourth brood per year in California (Barnes 1944). The different larval stages overlap least in the first brood but larvae of all five stages occur together in late season. The larvae are small and most easily killed with insecticides during the following three periods of the growing season: May 15 to May 25, July 1 to July 15, and August 15 to September 1. These three periods vary from year to year, depending on climatic conditions. The moths of the second and third flights tend to lay their eggs on leaves rolled by larvae of previous generations. This tends to concentrate the attack on the vines on which eggs were laid by the first-flight moths in May. Sometimes, however, the moths of the second and third flights lay eggs in previously uninfested vineyards. A serious infestation may result.

Prior to 1954 the grape leaf folder was generally serious only every third or fourth year. Although egg parasites and parasites of the pupae (Donohoe 1937) are known to

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occur, it has been generally accepted that the larval parasite *Microbracon cushmani* Mues. was responsible for keeping this pest in check. Since 1954, effective parasitism has occurred only locally and infrequently in much of the southern San Joaquin Valley. Not until 1959 was parasitism of the larvae general over a large enough area to make treatments for the first and second broods unnecessary. Chemical control had been applied over most of this area during the previous growing season.

Standard lead arsenate or cryolite has been used for many years. Stafford & Jensen (1957) reported the ineffectiveness of cryolite dust and listed as promising insecticides: parathion, endrin, methyl-parathion, and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate). Of lower effectiveness as dusts were: Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), ethion, Phostex® (a mixture of bis (dialkyloxyphosphinothioyl), and a pyrethrum-rotenone mixture. Jensen (1958) noted the importance of using a persistent insecticide for control of first-brood larvae. He listed the desirable qualities of insecticides for use on the grape leaf folder and reported Sevin® (1-naphthyl-*N*-methyl carbamate) a very promising material.

METHODS AND MATERIALS.—The results of applications of insecticides to control grape leaf folders were measured in numbers of leaf rolls. When insecticides were applied for the control of the first-brood larvae, then leaf rolls were counted in late June or early July, when feeding by larvae of this brood was almost completed. Usually only insecticides having long persisting residues were used to control first-brood larvae. The persistent effects of chemicals used against the first-brood larvae were measured by a second count of leaf rolls made in mid-August, when feeding of the larvae of the second brood was almost completed. Pretreatment and posttreatment leaf-roll counts were made to measure the effects of insecticides applied to control larvae in the second or third broods. In some cases where second-brood larvae were controlled, the posttreatment counts showed fewer rolls than the pretreatment counts. This lower count of rolls was the result of a good control of larvae and growth of new leaves, which hid the old leaf rolls. Counts were always made as the observer walked between vine rows without disturbing the foliage to find hidden leaf rolls.

Test plots in commercial vineyards were from one to four rows wide. The row on which counts were made in untreated plots was protected by at least one untreated guard row on either side. Dust plots were at least three rows wide. Sprays were applied using a power sprayer equipped with fixed spray-booms or by means of hand-held spray guns. Dusts were applied with power dusters operated by the farmer or by a commercial operator. The materials used were either formulations of experimental materials supplied by the manufacturer or formulations that were available commercially. The actual materials used and other information on sample size and number of replicates are given in the tables of results.

RESULTS.—During 1958 and 1959, field tests were made to control grape leaf folder larvae. Control of each of the first three broods was investigated separately and none of the experimental plots received more than one application of insecticide. Three tests were made on first-brood larvae. In one test (table 1) the first counts showed that the Sevin dust was the most effective treatment followed by sprays of endrin, Sevin, and acid lead arsenate plus malathion. The results of a second count made 49 days later showed that endrin sprays had the most persistent effect followed by the Sevin dusts. In the interval between counts, July 2 to August 20, the numbers of leaf rolls increased 46-fold on untreated vines. In this trial, lead arsenate showed less persistent effect than it had shown in other tests on record. The poor persistent effect of Sevin spray was surprising in the light of previous experiences with this material. This poor persistence may be attributed to the casein-lime spreader that was inadvertantly added to the Sevin spray. The detrimental effect of this spreader on the action of Sevin was later demonstrated in the laboratory by Moorefield (1958). Incidentally, Sevin dust and malathion spray showed better control of grape leafhoppers (*Erythroneura elegantula* Osb.) than the endrin spray (data not given). The Sevin-casein-lime spray was not so effective in controlling leafhoppers as the Sevin dust.

Table 1.—Per cent reduction in leaf rolls on Alicante grapes at Sanger, California. 1958.

MATERIALS[a] PER 100 GALLONS OR POUNDS OF DUST PER ACRE	ACTIVE INGREDIENT PER ACRE (LB.)	REDUCTION OF LEAF ROLLS[b] (%)	
		July 2	Aug. 20
Sevin 50W, 1 lb.[c]	1.1	74	45
Sevin 5%–sulfur 50%, 35 lb. per acre	1.8	86	72
Lead arsenate, 4 lb., Malathion 25W, 2 lb.[c]	8.9 1.1	53	45
Endrin 50W, 0.5 lb.[c]	.55	80	87

[a] Applied May 27 and 28.
[b] As compared with untreated vines. Average of 3 replicates of 100 vines each.
[c] All sprays contained 4 pounds wettable sulfur and 1 lb. casein-lime spreader per 100 gallons.

Table 2 gives the results of another test made in 1958 for control of first-brood larvae. In this case all treatments showed good persistent effect. The lead arsenate spray showed a degree of persistence consistent with results obtained in previous tests. Between the dates of the first and second counts, June 25 to August 12, the numbers of leaf rolls increased 12-fold on untreated vines.

The results of treatments to control first-brood larvae at Exeter in 1959 are given in table 3. In contrast to ob-

Table 2.—Per cent reduction in leaf rolls on Emperor grapes at Exeter, California. 1958.

MATERIALS[a] PER 100 GALLONS OR POUNDS OF DUST PER ACRE	ACTIVE INGREDIENT PER ACRE (LB.)	REDUCTION OF LEAF ROLLS[b] (%)	
		June 25	Aug. 12
Lead arsenate, 4 lb.[c]	11.6	90	92
Sevin 50W, 1 lb.[c]	1.4	98	96
Sevin 5%–sulfur 50% (18 lb. per A.)	0.9	93	86

[a] Applied May 23.
[b] As compared with untreated vines. Average of 5 replicates of 80 vines each.
[c] All sprays contained 4 oz. Triton B 1956 and 2 lb. of wettable sulfur per 100 gallons.

Table 3.—Per cent reduction in leaf rolls on Emperor grapes at Exeter, California. 1959.

Materials per 100 Gallons[a]	Active Ingredient per Acre (lb.)	Reduction of Leaf Rolls[b] (%)	
		June 15	Aug. 18
Sevin 50W, 1 lb.	1.0	99.1	95.0
Endrin 50W, ½ lb.	0.5	99.9	97.5
Bacillus thuringiensis,[c] 2 lb.		98.4	83.9

[a] All sprays applied May 15 and contained 2 pounds wettable sulfur per 100 gallons.
[b] As compared with untreated checks. Average of 5 replicates of 80 vines each.
[c] Rohm & Haas Co., 25 billion spores per gram.

servations made in 1958, there was only an 8.3-fold increase in leaf rolls from the end of the first brood to the end of the second brood (June 15 to August 18) on untreated vines. Excellent control of first-brood larvae was obtained with sprays of Sevin, endrin, and *Bacillus thuringiensis* spores. Persistent effects were very good with Sevin and endrin but definitely inferior with the *Bacillus* spores.

Control of second- and third-brood larvae was measured by changes in numbers of rolls per vine or per half-vine, *i.e.*, before and after treatment. In all cases the south halves of the vines were used to determine the numbers of rolls per half-vine. Table 4 gives the results of treatments to control second-brood larvae at Exeter in 1959. Both Sevin and endrin dusts gave excellent control. A *B. thuringiensis* dust did not control the grape leaf folder. A count made on July 7 showed 3.3, 0.9, and 2.4 larvae per roll for treatments of Sevin, endrin, and *B. thuringiensis*, respectively. Both larval and leaf-roll counts indicated better control with endrin than with Sevin.

The results of tests to control third-brood larvae are given in table 5. In these tests both Sevin and parathion dusts gave adequate control. Though adequate, the Sevin-Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) combination dust was not so satisfactory as Sevin without Kelthane. Table 6 gives results of treatments for control of third-brood larvae with previously untried chemicals as compared with insecticides previously shown to be effective. The dust of Bayer 22408 (*O,O*-diethyl *O*-naphtalimido phosphorothioate) was freshly prepared from a wettable powder containing 50% of the active ingredient. The dusts of dimethoate, Sevin, Diazinon, and Bayer 22408 gave very good control while Methyl-Trithion® (*S*-(*p*-chloro-phenylthio) methyl dimethyl phosphorodithioate), Kepone® (decachloroöctahydro-1,3,4-methano-2*H*-cyclobuta[cd]pental-en-2-one) and *B. thuringiensis* dusts were not satisfactory in these preliminary

Table 4.—Per cent change in numbers of leaf rolls on Emperor grapes at Exeter, California. 1959.

Dust Material[a] and Pounds per Acre	Active Ingredient per Acre (lb.)	Average Number Rolls/ Vine on June 30	Per Cent Increase (+) or Decrease (−) in Number of Rolls[b] from June 30 to Aug. 18
Sevin 5%–sulfur 25%, 22 lb.	1.1	21.5	− 21
Endrin 1.5%–sulfur 25%, 25 lb.	0.38	14.2	− 39
Bacillus thuringiensis,[c] 25 lb.		13.9	+142

[a] Applied June 30.
[b] Plots of 80 vines, not replicated.
[c] Thuricide 10—Stauffer Chemical Co. 300 million viable spores per gram.

Table 5.—Per cent change in numbers of leaf rolls on Thompson grapes at Fresno, California. 1958.

Dust Material[a]	Active Ingredient per Acre (lb.)	Average Number Rolls/¼-Vine on Aug. 21	Per Cent Increase in Rolls[b] from Aug. 21 to Sept. 17
Sevin 5%–Sulfur 25%	1.1	6.6	38.0
Sevin 5%–Sulfur 25%-Kelthane 2%	1.1 0.4	4.9	53.9
Parathion 2%	0.4	11.6	57.0

[a] Applied August 21 at 22 pounds per acre.
[b] Average of 3 replicates of 61 vines each.

tests. The grapes on vines treated with Methyl-Trithion and Bayer 22408 were harvested before final leaf-roll counts were made. It appeared that in the process of harvesting many leaves were removed from the vines. Thus the leaf-roll counts for vines dusted with these two materials probably should have been greater than were observed. In these tests the grape leafhoppers were controlled by Sevin, dimethoate, and Diazinon (data not given). Methyl-Trithion gave partial control of leafhoppers while Bayer 22408, Kepone, and the *B. thuringiensis* dust were not effective.

Analyses for Diazinon and Sevin were made by Pesticide Residue Research at Davis, California. A 3% Diazinon dust was applied by a power duster to Tokay grapes at the rate of 25 pounds per acre on August 25, 1958. Samples of berries were taken on 0, 3, 5, and 7 days after harvest. The corresponding residues in p.p.m. were 0.027, 0.012, less than 0.002, and less than 0.002. A 5% Sevin dust was applied by power duster to different plots of Thompson grapes on each of six dates at the rate of 20 pounds per acre. All plots were sampled on September 3, 1958, when the last application of dust was made. The dates when the dust was applied, days before sampling, and corresponding residues of Sevin in p.p.m. were as follows: August 20 (14 days) 0.11, August 27 (7 days) 0.30, August 30 (4 days) 0.52, September 1 (2 days) 0.75, September 2 (1 day) 1.7, and September 3 (0 day) 1.6.

The two chemicals endrin and Sevin have given good control of grape leaf folder larvae in almost all field tests conducted over a period of 3 years. Endrin appears to have no repellent effect on the larvae. Larvae make rolls on endrin-treated leaves and start to feed. After a little

Table 6.—Per cent change in numbers of leaf rolls on Thompson grapes at Sanger, California. 1959.

Dust Material[a] and Pounds per Acre	Active Ingredient per Acre	Average Number Rolls/ ¼-Vine on Aug. 26	Per Cent Increase (+) or Decrease[b] (−) in Leaf Rolls from Aug. 26 to Sept. 15
Diazinon 2% 33 lb.	0.7	17.1	+ 10.3
Sevin 5% 35 lb.	1.8	11.3	+ 6.1
Dimethoate 2% 46 lb.	0.9	16.1	− 3.9
Kepone 10% 28 lb.	2.8	13.3	+135
Methyl-Trithion 2% 44 lb.	0.9	6.2	+102
Bayer 22408—6% 46 lb.	2.8	14.6	− 47.7
Bacillus thuringiensis[c] 34 lb.		11.4	+153
Untreated[d]		7.1	+232

[a] Applied August 26.
[b] Unreplicated plots of 115 vines each.
[c] Thuricide 10—Stauffer Chemical Co. 300 million spores per gram.
[d] Average of three plots.

feeding the larvae become sluggish and remain quiet until they die. This action of endrin leaves dead larvae in rolls where they may be easily found. Larvae are repelled by deposits of Sevin on grape leaves. They tend to fall off the leaves. Very young larvae are killed by contact with Sevin. So far, the increase of spider mites in vineyards treated with Sevin seems to be negligible. Sevin also controls leafhoppers. It is relatively safe to use, and dusts may be applied at recommended dosages up to harvest without exceeding the tolerance. *Bacillus thuringiensis* is a promising control for grape leaf folder larvae, though less persistent than endrin, Sevin, or lead arsenate. Correct dosages, especially for second- and third-brood larvae, have not yet been determined.

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Some Effects of Age, Preimaginal Habitat, and Adult Food on Susceptibility of Boll Weevil to Certain Insecticides[1]

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ABSTRACT

Experiments were conducted to determine the toxicity of Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), BHC, and toxaphene to the boll weevil under certain conditions of preimaginal habitat, adult food source, and weevil age. There was a 48- and 61-fold difference in the LD-50 of BHC and toxaphene, respectively, between the easiest to kill group (square-reared, bloom-fed, 2-day-old) and the hardest to kill group of weevils (boll-reared, boll-fed, 9-day-old). The LD-50 values were relatively constant in all tests using Guthion.

The susceptibility of boll weevils to BHC and toxaphene was influenced to a greater extent by the adult food source than the age of the weevil or its preimaginal habitat.

Based on LD-50 values, the order of susceptibility of the boll weevil to BHC and toxaphene was: boll-reared < square-reared, square-fed < bloom-fed, boll-fed < square-fed, and 9-day-old < 2-day-old weevils.

Certain chlorinated hydrocarbon insecticides are often less effective against the boll weevil, *Anthonomus grandis* Boh., late in the season than early (Rainwater & Gaines 1951, Gaines & Mistric 1952). This natural tolerance of an insect to insecticides has been confused with heritable resistance. Gaines & Mistric (1952) found that boll-reared weevils were considerably more difficult to kill than square-reared weevils, and Blum *et al.* (1959) reported that weevils became progressively more difficult to kill from the time of emergence up to 5 days of age.

Other factors implicated in contributing to natural tolerance were climate (Gaines & Dean 1949, 1950, Gaines & Mistric 1951, 1952, Mistric & Gaines 1953, 1954), source of adult food (Brazzel *et al.* 1957), and diapause (Brazzel 1959).

The studies reported herein were an attempt to explore further some of the effects of weevil age, preimaginal habitat, and adult food on the susceptibility of the weevil to Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl)phosphorodithioate), BHC, and toxaphene; and to establish by using varied combinations of these factors both the single effect of that factor and the effect of various combinations of the factors previously mentioned.

METHODS AND PROCEDURES.—During the summer of 1958, cotton squares and bolls infested by boll weevils were collected in the field from an insecticide-free area at Auburn, Alabama. These were brought into the laboratory where the squares were placed in large cardboard boxes and the bolls were placed in screen wire cages. As the weevils emerged, they were kept segregated according to their preimaginal habitat (squares or bolls). The weevils were held in quart cylindrical cardboard containers until they reached the ages of 2 or 9 days. Tests were conducted using weevils from each preimaginal habitat at 2 days of age and at 9 days of age. Weevils for a specific test were fed from only one food source, *i.e.*, blooms, squares, or bolls. The first test was conducted in July using square-reared, square-fed, 2-day-old weevils. In September, at the end of the test period, the first test was duplicated for the purpose of comparison. Combinations of the above factors included in this study are given in table 1.

Acetone solutions of technical Guthion, BHC, and toxaphene were used in the experiments. The various strength solutions used were made by weighing out a given amount of the technical insecticide and making a relatively concentrated stock solution. From this, the various working solutions were made by dilution. One

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Table 1.—Toxicity of toxaphene, BHC, and Guthion to boll weevils having various combinations of preimaginal habitat, age, and adult food.

Food	Age of Weevils (days)	LD-50 and Fiducial Limits in μg./Gm. Toxaphene	BHC	Guthion
		Square-reared		
Blooms	2	81.1± 31.2	17.6± 6.2	1.5±0.5
Squares	2[a]	140.9± 58.4	23.3± 9.2	2.6±0.8
Squares	2[b]	153.2± 53 9	46.1± 16.7	2.3±0.8
Squares	9	542.1± 194.9	123.0± 44.0	3.6±0.9
Bolls	2	2,099.0± 707.0	201.4± 68.8	4.9±1.9
Bolls	9	4,385.0±1,544.5	633.9±258.2	3.8±1.3
		Boll-reared		
Squares	2	330.4± 102.3	43.0± 14.0	1.5±0.6
Squares	9	1,067.0± 349.8	292.4±105.7	2.8±1.0
Bolls	2	4,932.0± 153.0	389.9±126.6	2.6±0.9
Bolls	9	4,943.0±1,537.5	849.2±292.2	2.9±1.0

[a] July.
[b] September.

microliter of the insecticide-acetone solution was applied to the dorsal surface of the prothoracic segment of each weevil. The same volume of acetone was applied to other weevils to serve as checks.

For each dosage level, 10 weevils of approximately the same size were anesthetized with CO_2, weighed, treated, and placed in half-pint cardboard cartons with screen wire tops. Each treatment was replicated 10 times. The weevils were fed immediately after treatment and at 24-hour intervals thereafter on the same food source as prior to treatment. Mortality counts were made 72 hours after treatment.

The data were analyzed statistically by the method of Bliss (1938) and are presented as LD-50 values and as dosage-mortality curves.

Results and Discussion.—Preimaginal habitat, adult food source, and weevil age significantly influenced boll weevil susceptibility to toxaphene and BHC but had considerably less effect on weevil susceptibility to Guthion (table 1 and figs. 1, 2, and 3).

The one factor that exhibited the greatest effect on boll weevil susceptibility to the three insecticides tested was the adult food source. Square-reared, boll-fed, 2-day-old weevils were 26 times more difficult to kill with toxaphene, 12 times more difficult to kill with BHC, and 3 times more difficult to kill with Guthion than square-reared, bloom-fed, 2-day-old weevils (table 1). Similar but less pronounced differences were noted between square-fed and boll-fed weevils in all tests with the three insecticides.

Age was also an important factor contributing to boll weevil susceptibility to insecticides. There was almost a 7-fold increase in the LD-50 of BHC to boll-reared, square-fed, 9-day-old weevils as compared with a comparable group of 2-day-old weevils; a 2-fold difference was noted with Guthion. Approximately a 4-fold increase in the LD-50 of toxaphene was noted for 9-day-old as compared with 2-day-old square-reared, square-fed weevils. Similar but less pronounced differences were noted between 2- and 9-day-old weevils with other combinations of factors (table 1).

Preimaginal habitat was the factor that least affected boll weevil susceptibility to the three insecticides tested. Preimaginal habitat had the greatest effect on weevil susceptibility to BHC; square-fed, 9-day-old weevils were three times more difficult to kill when the preimaginal habitat consisted of bolls as compared with squares. The LD-50 of toxaphene to boll-reared weevils was usually about twice that of square-reared weevils. Preimaginal habitat had little effect on the toxicity of Guthion to the boll weevil.

Based on the LD-50, boll-reared, boll-fed, 9-day-old weevils were 61, 48 and 2 times more difficult to kill with toxaphene, BHC, and Guthion, respectively, than square-

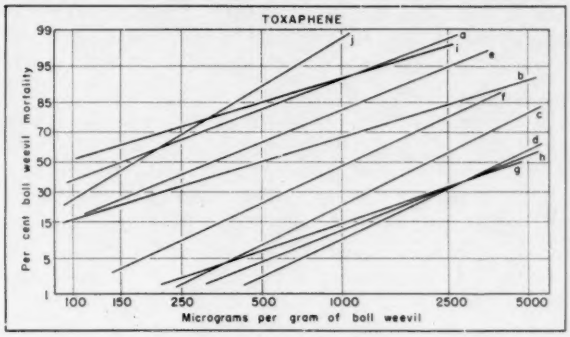

Fig. 1.—Dosage mortality curves of toxaphene to the boll weevil.[2]

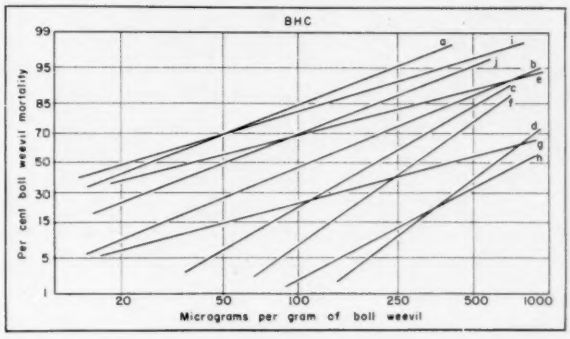

Fig. 2.—Dosage mortality curves of BHC to the boll weevil.[2]

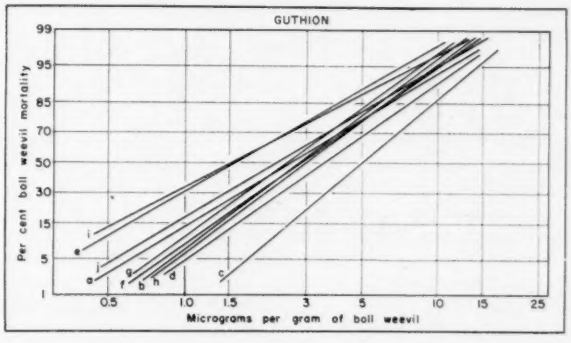

Fig. 3.—Dosage mortality curves of Guthion to the boll weevil.[2]

[2] Dosage mortality curves of insecticides to the boll weevil: (a) square-reared, square-fed, 2-day-old (July-collected); (b) square-reared, square-fed, 9-day-old; (c) square-reared, boll-fed, 2-day-old; (d) square-reared, boll-fed, 9-day-old; (e) boll-reared, square-fed, 2-day-old; (f) boll-reared, square-fed, 9-day-old; (g) boll-reared, boll-fed, 2-day-old; (h) boll-reared, boll-fed, 9-day-old; (i) square-reared, bloom-fed, 2-day-old; (j) square-reared, square-fed, 2-day-old (September-collected).

reared, bloom-fed, 2-day-old weevils. These toxicity differences with toxaphene and BHC were more pronounced with this combination of factors than any other combination studied. With Guthion there was a 3-fold difference between the easiest-to-kill group (square-reared, bloom-fed, 2-day-old) and the most-difficult-to-kill group of weevils (square-reared, boll-fed, 2-day-old). Boll-reared, boll-fed, 9-day-old weevils were 35 to 37 times more difficult to kill with either toxaphene or BHC than square-reared, square-fed, 2-day-old weevils while the toxicity of Guthion to both groups of weevils was about the same. Using square-reared, square-fed, 2-day-old weevils there was no significant difference in the susceptibility of July- and September-collected weevils to Guthion, BHC, or toxaphene.

Factors affecting boll weevil susceptibility to toxaphene, BHC, and Guthion ranked in decreasing order of importance were: adult food, age, and preimaginal habitat. The adult food source influenced weevil susceptibility to toxaphene more than to BHC; the least effect was noted with Guthion. Age affected boll weevil susceptibility to BHC more than to toxaphene. The least effect was noted to Guthion. Two-day-old weevils were generally more susceptible to the insecticides tested than were 9-day-old weevils. Preimaginal habitat had its greatest effect on the toxicity of BHC to boll weevils, less effect on toxaphene, and little or no effect on Guthion.

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Stored-Grain Insects and Their Control in New Jersey[1]

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ABSTRACT

A study initiated in New Jersey in 1955 showed a total of 53 species of insects found in and around stored-grain structures and included 36 species of Coleoptera, 5 species of Lepidoptera, 1 species of Psocoptera, 9 species of parasitic Hymenoptera, and 2 species of predacious Heteroptera.

Twenty-eight bins were treated at harvest in 1956 with pyrethrins emulsion at 1.0, 1.5, and 2.0 p.p.m., a pyrethrins dust at 1.5 p.p.m., malathion emulsion at 25.0 p.p.m., and malathion dust at 5.0 p.p.m. The most effective treatments were the pyrethrins dust and the malathion dust and emulsion. Twenty-one bins were treated at harvest in 1957 with a pyrethrins emulsion at 1.5 and 2.0 p.p.m., a pyrethrins dust at 1.5 p.p.m., and malathion dust at 5.0 p.p.m. A fumigant containing 3.5% ethylene dibromide, 10.0% ethylene dichloride, 10.0% carbon bisulphide, and 76.5% carbon tetrachloride was applied to seven bins 6 weeks after harvest. The latter treatment, plus malathion dust and the highest dosage of the pyrethrins emulsion, provided the most effective protection against insects.

Species such as the foreign grain beetle (*Ahasverus advena* Waltl) and a hairy fungus beetle (*Typhaea stercorea* (L.)) were abundant only in 1956 when the grain had an average moisture content of 14.0% at initial storage. The saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)) and the rusty grain beetle (*Laemophloeus ferrugineus* (Steph.)), especially the former, were abundant both years. The Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) was observed infesting wheat, corn, rye, and barley in the field.

The fumigant mixture applied at the manufacturer's recommendations, was also very effective in reducing heavy insect infestations in 10 bins of wheat in the fall of 1956.

Since the inception of the government loan program in 1938, more grain is being stored in New Jersey and problems related to grain storage have increased tremendously. Many of these problems have resulted from inadequate grain storage facilities, incomplete knowledge of the insects involved, and the absence of proven control measures. A project was initiated in 1955 to find the answers to some of these problems and the findings of the more important phases are herein reported.

INSECT SPECIES.—A total of 53 species of insects were recovered from in and around grain storages. The list, presented in table 1, includes 36 species of Coleoptera, 5 species of Lepidoptera, 1 species of Psocoptera, 9 parasitic species of Hymenoptera, and 2 predatory species of

[1] This study was conducted with funds from the Agricultural Marketing Service, United States Department of Agriculture, under contract number 12-25-010-101. The Agricultural Marketing Service neither restricts nor is responsible for the opinions or conclusions of the author.

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Table 1.—List of species found in and around grain storages in New Jersey, 1955–58.[a]

ORDER	FAMILY	GENUS AND SPECIES
Coleoptera	Bostrichidae	*Rhyzopertha dominica* (Fabr.)
	Bruchidae	*Bruchus brachialis* Fabr.
	Cryptophagidae	*Cryptophagus* sp.
		Henoticus sp.
	Cucujidae	*Ahasverus advena* Waltl
		Laemophloeus ferrugineus (Steph.)
		Laemophloeus pusillus (Schonh.)
		Oryzaephilus surinamensis (L.)
	Curculionidae	*Sitona hispidula* (F.)
		Sitophilus granarius (L.)
		Sitophilus oryza (L.)
	Dermestidae	*Anthrenus verbasci* (L.)
		Attagenus sp.
		Dermestes lardarius L.
		Trogoderma inclusum LeConte
	Lathridiidae	*Corticaria* sp.
		Lathridius minutus (L.)
		Microgramme ruficollis (Marsh.)
	Mycetophagidae	*Litargus sexpunctatus* Say
		Mycetophagus pleuripunctatus LeC.
		Mycetophagus sp.
		Typhaea stercorea (L.)
	Nitidulidae	*Carpophilus hemipterus* (L.)
		Carpophilus mutilatus Er.
		Carpophilus sayi Parsons
	Ostomidae	*Tenebroides mauritanicus* (L.)
	Ptinidae	*Ptinus fur* L.
		Ptinus hirtellus Sturm
	Staphylinidae	One unidentified species
	Tenebrionidae	*Alphitobius diaperinus* (Panz.)
		Alphitophagus bifasciatus (Say)
		Palorus ratzeburgi (Wiss)
		Tenebrio molitor L.
		Tenebrio obscurus F.
		Tribolium castaneum (Hbst.)
		Tribolium confusum Duv.
Lepidoptera	Gelechiidae	*Sitotroga cerealella* (Oliv.)
	Phycitidae	*Ephestia kuhniella* Zell.
		Plodia interpunctella (Hbn.)
	Pyralidae	*Pyralis farinalis* (L.)
	Tineidae	*Nemapogon granella* (L.)
Psocoptera	Lachesillidae	*Lachesilla pedicularia* L.
Heteroptera	Anthocoridae	*Lyctocoris campestris* (F.)
		Xylocoris galactinus (Fieber)
Hymenoptera	Bethylidae	*Cephalonomia tarsalis* (Ashm.)
		Cephalonomia waterstoni Gahan
		Plastanoxus sp.
	Braconidae	*Bracon hebetor* Say
	Eurytomidae	*Eurytoma* sp.
	Ichneumonidae	*Idechthis canescens* (Grav.)
	Proctotrupidae	*Cryptoserphus abruptus* (Say)
	Pteromalidae	*Habrocytus cerealellae* (Ashm.)
		Lariophagus distinguendus (Foerst.)

[a] Identifications were either confirmed or made by R. I. Sailer, B. D. Burks, K. V. Krombein, C. F. W. Muesebeck, L. M. Walkley, H. W. Capps, R. S. Beal, T. J. Spilman, L. L. Buchanan (deceased), E. J. Gerberg, G. B. Vogt, and R. E. Warner of the U. S. Department of Agriculture, J. F. G. Clarke of the United States National Museum, and K. M. Sommerman of the United States Public Health Service, Anchorage, Alaska.

Heteroptera. A series of specimens of *Mycetophagus* sp. (Mycetophagidae-Coleoptera) and *Henoticus* sp. (Cryptophagidae-Coleoptera) collected from damp, shelled corn are, according to taxonomists at the United States National Museum, either new or introduced species.

SAMPLING TECHNIQUE.—Approximately 5 minutes were spent searching for insects in the immediate vicinity of the bins. All bins were sampled with a 5-foot grain trier. Five vertical probes, one from each quadrant and one from the center, were drawn from each bin. A final probe was taken in the center of the bin with the trier horizontal and several inches below the grain surface. All probes were combined, placed in a plastic bag, and a 1,000-gram sample from it was screened in the laboratory for live insects, animal fecal pellets, and damage. The moisture content was checked by a Tag-Heppenstall moisture meter. Most bins were checked monthly from the time of treatment until the grain was removed.

PROTECTANT TREATMENTS.—In 1956, the following treatments were applied to approximately 40,000 bushels of wheat in 28 bins: 4 bins with a pyrethrins emulsion at 1.0 p.p.m.; 4 bins with a pyrethrins emulsion at 1.5 p.p.m.; 4 bins with a pyrethrins emulsion at 2.0 p.p.m.; 8 bins with a pyrethrins dust at 1.5 p.p.m.; 4 bins with malathion emulsion at 25.0 p.p.m.; and 4 bins with malathion dust at 5.0 p.p.m. Thirteen untreated bins were used as controls.

In 1957, the following protectant treatments were applied to approximately 35,000 bushels of wheat in 27 bins; 5 bins with a pyrethrins emulsion at 1.5 p.p.m.; 6 bins with a pyrethrins emulsion at 2.0 p.p.m.; 7 bins with a pyrethrins dust at 1.5 p.p.m.; 3 bins with malathion dust at 5.0 p.p.m.; and 6 bins with a fumigant. Seven untreated bins were used as controls.

Prior to all treatments, the bins were thoroughly cleaned and sprayed by the farmer with methoxychlor or malathion and, in a few instances, with DDT. All emulsions were applied to the grain at the auger or vacuvator with a 5-gallon back sprayer at the rate of 5 gallons per 1,000 bushels of wheat. Only Pyrenone O.F. 60-6 Emulsifiable Concentrate (containing 6% pyrethrins and 60% piperonyl butoxide) and Premium Grade Emulsifiable malathion were used, with the concentrations varied to give the desired dosage. The dusts were applied by hand at the auger or in the truck bed when the vacuvator was used. The synergized pyrethrum wheat protectant dust contained 0.08% pyrethrins and 1.1% piperonyl butoxide, whereas the malathion contained 0.5% premium grade toxicant on flour; both were applied to the wheat at the rate of 115 and 62.5 pounds of dust, respectively, per 1,000 bushels of wheat. The wheat was fumigated 6 weeks after harvest at 3 and 4 gallons of fumigant per 1,000 bushels of wheat per metal and wood bin, respectively. The fumigant contained the following formulation: 3.5% ethylene dibromide; 10.0% ethylene dichloride; 10.0% carbon bisulphide; and 76.5% carbon tetrachloride.

RESULTS.—The results of these treatments are presented in tables 2 and 3. The data were evaluated according to the number of actual bin-months of protection, the desired bin-months of protection, and the per cent

Table 2.—A comparison of various treatments and dosages in 1956 and in 1957 with the resultant protection expressed according to bin-months of protection and per cent effectiveness.

NO. BINS	TREATMENTS (P.P.M.)	BIN-MONTHS OF PROTECTION		PER CENT EFFECTIVENESS
		Actual	Desired	
	1956–57			
13	No treatment—controls	1.2	4.2	28
4	Pyrethrins emulsion, 1.0	2.5	3.8	66
4	Pyrethrins emulsion, 1.5	1.8	4.2	42
4	Pyrethrins emulsion, 2.0	3.0	4.7	63
8	Pyrethrins dust, 1.5	3.8	4.1	92
4	Malathion emulsion, 25.0	3.8	4.2	89
4	Malathion dust, 5.0	3.8	4.2	95
	1957–1958			
7	No treatment—controls	1.7	6.0	28
5	Pyrethrins emulsion, 1.5	5.0	6.0	83
6	Pyrethrins emulsion, 2.0	6.0	6.0	100
7	Pyrethrins dust, 1.5	5.1	5.8	88
3	Malathion dust, 5.0	6.0	6.0	100
6	Fumigation	4.5	4.5	100

Table 3.—Comparative abundance of insects in 1-quart random probe samples of treated and untreated wheat in 1956–57 and 1957–58 in New Jersey.

	1956–57							1957–58				
	Pyrethrins				Malathion			Pyrethrins			Malathion	
	Dust	Emulsion			Dust	Emulsion		Dust	Emulsion		Dust	
Species	1.5[b]	1.0	1.5	2.0	5.0	25.0	Untreated	1.5	1.5	2.0	5.0	Untreated
Oryzaephilus surinamensis	0.5	2.3	16.5	21.0	0	0	65.5	0	5.7	0.8	0	121.5
Laemophloeus spp.[a]	0	.3	0	0	.3	0	21.8	0	0	0	0	12.5
Tribolium castaneum	0	0	0	0	0	0	.1	1.3	0	0	0	0
Ahasverus advena	1.9	4.3	0	0	0	0	44.3	0	0	0	0	1.9
Typhaea stercorea	.8	.7	0	.7	2.2	.3	18.3	0	0	.2	0	.7
Plodia interpunctella	.5	1.3	0	0	0	0	1.8	0	0	.2	0	4.1
Beetle larvae	1.5	6.0	2.5	11.8	3.8	1.3	58.8	.2	0	0	0	81.2
Miscellaneous adults	.4	0	0	.3	0	0	2.3	0	1.0	.2	0	15.0[c]
Totals	5.5	15.0	19.0	33.8	6.3	1.5	212.8	1.5	6.7	1.4	0	237.0

[a] Predominantly the rusty grain beetle (*L. ferrugineus*) with some flat grain beetle (*L. pusillus*) present.
[b] Dosage in parts per million of active ingredient.
[c] Predominantly hymenopterous parasites.

effectiveness of the treatments. The number of actual bin-months of protection was computed by dividing the total number of months of protection for a group of bins receiving the same treatment by the number of bins in that group. A treatment was considered effective and providing protection until the wheat would be designated as "weevily" under the U. S. Grain Standards. They state that wheat shall be considered weevily if the portion of the sample used for the determination of dockage contains any of the following conditions: (a) more than one live weevil, (b) one live weevil, and the remainder of the original sample contains any live weevil or other live insects injurious to stored grain, (c) one live weevil and any other live insects injurious to stored grain, (d) does not contain any live weevil, but does contain 5 or more other live insects injurious to stored grain, or (e) a considerable number of live Angoumois moths or other live moths are present in, on, or about the lot of wheat offered for inspection. The desired bin-months of protection was obtained by dividing the total number of months the grain in a group of bins receiving the same treatment was stored, by the number of bins in that group. The per cent effectiveness was derived by dividing the actual bin-months of protection received by the bin-months of protection desired. It was necessary to adjust the actual bin-months of protection because with some treatments, although the grain was provided perfect protection, it was moved out a month earlier than with other treatments, or when studies, as in the fumigation, were initiated a month later than other treatments. These data show that the most effective materials in 1956 (table 2) were malathion dust, pyrethrins dust, and malathion emulsion, whereas in 1957 (table 2) the most effective materials were a pyrethrins emulsion at 2.0 p.p.m., malathion dust, and fumigation. In data not shown, most of the treatments in 1956 and all of those in 1957 provided excellent protection against larvae and adults of the Indian-meal moth, *Plodia interpunctella* (Hbn.).

The comparative abundance of the more common stored-grain insects in the various treatments and control in 1956–57 and 1957–58 is presented in table 3. The data show a great deal of variation in the abundance of species between the two years, which is probably closely correlated with the differences in the moisture content of the grain. The average moisture content of the grain at initial storage in 1956 and in 1957 was approximately 14.0 and 12.2%, respectively. In both years the saw-toothed grain beetle was the most abundant species in many of the treatments and in the untreated controls. The foreign grain beetle and hairy fungus beetle were very abundant in 1956 but much less so in 1957 when the grain was much drier.

Table 4.—Results of fumigation of 10 bins of stored-wheat at 3 and 4 gallons/1,000 bushels of wheat per metal and wood bin, respectively.

Number of Live Insects in 10 Bins[a]									
Pretreatment Counts			One-Week Counts				One-Month Counts		
Larvae	Adults	Total	Larvae	Adults	Total	Control (%)	Larvae	Adults	Control (%)
Treated bins									
480	808	1,288	3	41	44	97	0	23	98
Untreated bins									
9	31	40	7	35	42	—	0	65	—

[a] Based on 1-quart probe samples. Predominant insects were the saw-toothed grain beetle, foreign grain beetle, rusty grain beetle, and hairy fungus beetle.

FUMIGATION.—In addition to the fumigation of grain 6 weeks after harvest, fumigation was also used to curtail insect infestations causing "hot spots" in grain during the fall of 1956. Ten bins were fumigated with the same mixture that was used in the protectant treatments at 3 and 4 gallons per 1,000 bushels of wheat per metal and wood bin, respectively. The fumigant was evenly distributed over the surface of the wheat with a sprinkling can. The bins were sampled prior to treatment, at 1 week, and at a month following treatment. The results (table 4) indicate excellent immediate reductions of the heavy insect populations.

FIELD INFESTATION STUDIES.—Many samples were taken both years from wheat, rye, barley, corn, and oats in the field just prior to harvest. These samples were sealed in plastic bags and examined several months later for insect emergence. The Angoumois grain moth, *Sitotroga cerealella* (Oliv.), was found infesting samples of corn, wheat, and barley in 1956 and only rye in 1957. The degree of infestation appeared to vary with the severity of the preceding winter, with the climatic conditions in the spring and early summer, and with the distance the samples were taken from potential sources of infested stored grain or refuse.

House Fly Control in Pig Parlors[1]

EDWARD C. BURNS[2] and W. A. NIPPER[3]

ABSTRACT

White 16-ply cotton cords were soaked in 1% ronnel, 25% Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6 pyrimidinyl) phosphorothioate) and 25% Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) emulsifiable concentrates, allowed to dry, and hung from the ceiling or rafters in pig parlors. Sufficient 18-inch drops were used to provide 28.5 feet of vertical cord per 100 sq. ft. of floor space. House fly (*Musca domestica* L.) densities were determined immediately prior to and at weekly intervals following cord placement. Satisfactory control was being obtained with all materials in the test after 10 weeks.

The most important insect problem associated with the pig-parlor method of swine production in Louisiana is house fly (*Musca domestica* L.) control. When pig parlors are properly constructed, and operated, the breeding of flies on the premises is prevented, but large numbers of adult flies that are attracted to the area by the odors arising from the installation pose a problem. This problem extends throughout the entire fly season, since a swine-breeding program designed for continuous production and marketing of hogs is normally employed by farmers who utilize pig parlors.

A pig parlor consists essentially of a series of pens that are used to separate and confine swine of different ages and weights. In most cases only a portion of the structure is covered; the remainder serves as an open runway for the swine. The floor of the installation is concrete and is sloped, so that the droppings can be washed into ditches that lead to septic tanks or in some cases to nearby streams or bayous.

The effective use of cords impregnated with chlorinated hydrocarbon insecticides for control of house flies has been reported by Baker *et al.* (1947) and Chow & Thevasagayam (1953). The use of strings treated with the organic phosphates parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) has been reported by Maier & Mathis (1955), Kilpatrick (1955), Kilpatrick & Schoof (1956) and Schoof & Kilpatrick (1957). Tests using ronnel in a similar manner have been reported by Dry & Burns (1959), and Kilpatrick & Schoof (1959). Research was undertaken by the Louisiana Agricultural Experiment Station in 1958 to determine: (1) if the insecticide-impregnated-cord method of adult fly control would be effective when used under conditions normally found in pig parlors, and (2) the relative effectiveness of Diazinon, ronnel, and Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) when used as the impregnating material.

EXPERIMENTAL PROCEDURE.—White 16-ply cotton cord was used in all pig parlors in the study. To determine the amount of treated cord needed in a building the following formula was used:

$$\frac{\text{Building width}}{3} \times \text{Building length} + 3$$

This calculation gives the total lengths of the horizontal and vertical cords required. Vertical drops 18 inches long at 18-inch intervals on a horizontal cord provide 28.5 feet of vertical cord per 100 square feet of floor space. The horizontal cord with the vertical drops was soaked in emulsifiable concentrates of the insecticides being tested. Ronnel was used at 1% and Diazinon and Bayer 29493 were used at 25% emulsifiable concentrates. The cords were soaked 10 minutes in the solutions, dripped dry, then hung from the ceiling or rafters in rows 3 feet apart beginning 1½ feet inside the overhang. House fly densities were determined with the use of a Scudder (1947) grill. An average grill count was used in arriving at fly densities. Counts were made immediately prior to and at weekly intervals following string placement.

RESULTS.—The results of the test are shown in figure 1. Satisfactory control still was being obtained with all materials in the test after 10 weeks. Data from this study indicate that the treated-cord method will provide effective fly control around pig parlors.

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[1] Accepted for publication January 11, 1960.

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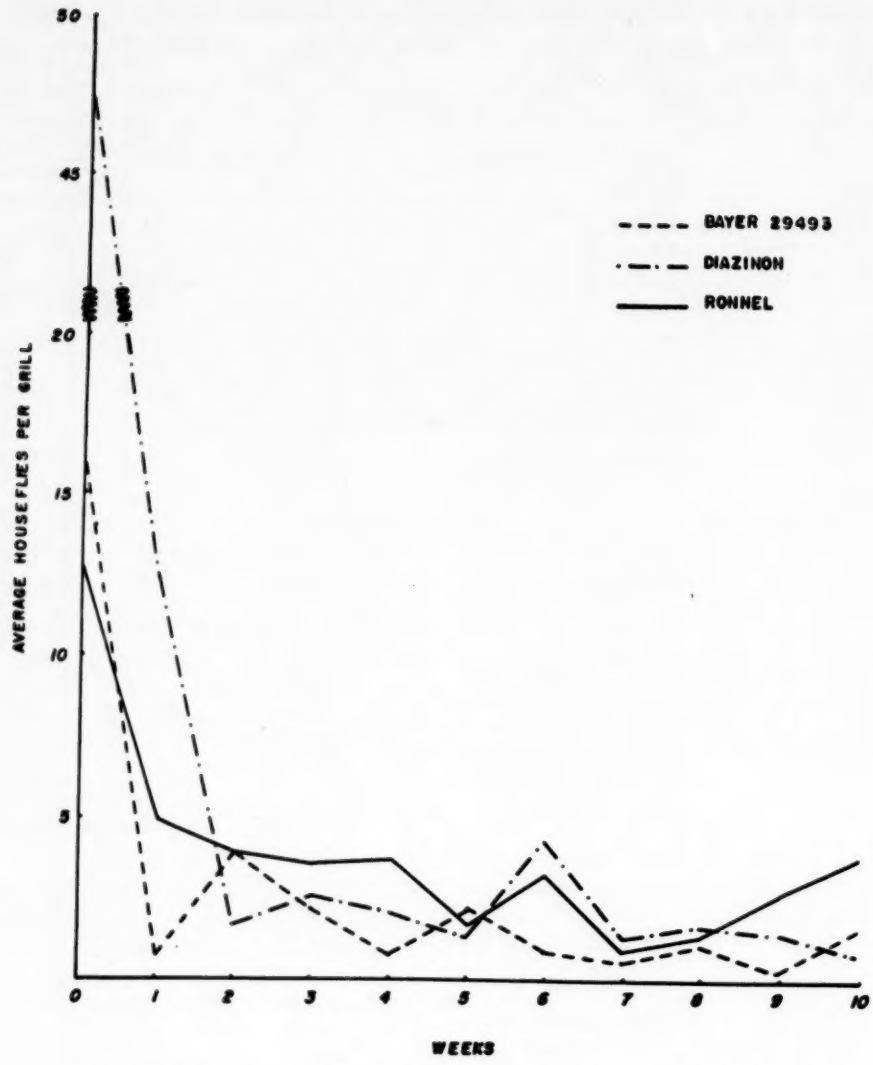

FIG. 1.—Fly populations following installation of insecticide-treated strings.

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Systemic Insecticides for Cattle Grub Control[1,2]

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ABSTRACT

Four animal systemic insecticides: ronnel; Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), also known as Bayer 21/199; Dowco 109 (phosphoramidothioic acid, methyl-*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ester); and dimethoate, also known as Am. Cyanamid 12880, were investigated for cattle grub control in Kansas during 1957–58. Results showed that November treatment of cattle from the Southern United States with Co-Ral and dimethoate greatly reduced the number of grubs in the backs of the animals but failed to rid the animals of migrating grubs. Dowco 109 as a spray gave a fair reduction of both the encysted and the migratory grubs, as did also the Dowco 109 used as a bolus and as a feed additive in December. September treatments of native cattle with Dowco 109 and Co-Ral sprays gave no appreciable control whereas the Dowco 109 as a bolus gave excellent control. Dowco 109, used as a feed additive on native cattle, gave very favorable results. Ronnel, used as a bolus on native cattle at monthly intervals from June through November, excluding October, greatly reduced the grubs in all treatments except August. A low-level feeding of ronnel for 79 days had no apparent effect upon grub control. No toxic symptoms were noted in any of the cattle treated.

Efforts of numerous workers have resulted in effective control of cattle grubs, *Hypoderma* spp., through the use of systemic insecticides. Since 1956 the technology in this field has rapidly progressed so that Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) and ronnel are now commercially available. Other systemic insecticides are also under investigation. The purpose of this paper is to present additional data so that timing and dosage of these insecticides can be more effective.

Reported are results with four animal systemics in Kansas during 1957 and 1958. Since numerous variables in these tests were involved, each is reported as a separate experiment.

The following procedure was used except when otherwise indicated: Ronnel as a bolus was administered at 110 mg./kg. body weight. In all spray tests, a pressure of 300 pounds and a No. 5 nozzle plate were used. Co-Ral, also known as Bayer 21/199, spray was formulated from a 25% wettable powder and the Dowco 109 (phosphoramidothioic acid, methyl-*O*-(4-*tret*-butyl-2-chlorophenyl) *O*-methyl ester) spray from either a 50% by weight emulsifiable concentrate or a 25% wettable powder at approximately 1 gallon per animal with thorough coverage of each animal. Dowco 109 boluses were used at approximately 15 mg./kg. body weight. Dimethoate, also known as Am. Cyanamid 12880, sampled as oblets, was used at the rate of 10 mg./kg. body weight. Dowco 109, sampled as a 10% pre-mix in wheat bran; and ronnel, sampled as a 10% pre-mix in soybean meal, were used for the feeding tests. All mixing of the pre-mix with a protein supplement was done by Western Star Mill, Salina, Kansas. The formulating process consisted of mixing the amount of systemic carrier needed with 100 pounds of supplement in a 500-pound shell blender for 10 minutes. This pre-mix was then brought to the required amount with the addition of more protein supplement and mixed for another 10 minutes in a Howell 5-ton mixer.

Animals receiving the medicated feed were fed on the assumption that all animals of one lot would eat the same amount, although some undoubtedly ate more than others.

The number of animals treated and the number counted for grubs were not always identical because some were consigned for sales or shows while others escaped the holding chute or were shipped before counts could be made.

In most experiments, animals were individually identified and grub counts made monthly from November to April on cattle from the Southern United States and from December to April on Kansas-bred cattle. Grubs were located by palpation and the location plotted on modified Riedel charts (Riedel *et al.* 1955).

EXPERIMENT 1.—This study was designed to obtain information on the best time for treatment in Kansas.

Three ecologically comparable herds of registered Hereford calves, ranging from 150 to 390 pounds, located in pastures at Herington, Kansas, were treated. One lot of 30 was treated June 12, 1 lot of 29 on July 15, and a lot of 27 on August 14, 1957. Boluses containing 10 gm. of ronnel were used. Thirty-two head in another pasture, the same age and weight, were left untreated.

The calves were removed from pasture in October 1957, weaned and shipped to various locations in Kansas, making it impossible to follow all of them throughout the study period. Of the lots, 18, 12, 3, and 17 head, respectively, were available for grub counts in December 1957, and March 1958.

The animals were weighed on the first treatment date, June 12, and October 10 when they were removed from pasture. The animals were on a regular creep ration, although grass was plentiful.

Results.—Table 1, based on the total number of grubs found, shows that the 18 animals remaining from the June treatment had a reduction of 97.9% in grubs, the 12 from the July treatment a 92.7% reduction and the three left from the August treatment a 71.9% reduction. The numbers infested were 3, 3, 2, respectively, while the untreated had 11 animals infested.

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[2] Portion of a dissertation to be presented by the senior author in partial fulfillment of the requirements for the degree Doctor of Philosophy in Entomology from the Graduate School, Kansas State University, Manhattan.

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Table 1.—Cattle grub control and 113-day weight gain on Hereford bull and heifer calves on a pasture creep feed ration.[a]

Date of Treatment	No. Animals	Average Number of Grubs/ Animal	No. Animals Infested	Grub Reduction (%)	Average Weight Gain (Lb.)	Average Difference Over Untreated (Lb.)
Untreated	17	9.6	11		221.5	
June 12	18	0.2	3	97.9	236.4	+14.9
July 15	12	0.7	3	92.7	213.8	− 7.7
August 14	3	2.7	2	71.9	240.0	+18.5

[a] Treated with ronnel as a bolus at 110 mg./kg. body weight.

The average changes in weight of the treated animals compared with the untreated were +14.9 pounds for the June treatment, −7.7 for the July treatment, and +18.5 for the August treatment.

EXPERIMENT 2.—This experiment was originally designed to control horn flies (*Siphona irritans* (L.)), using a low level of ronnel in the feed beginning July 17, for 79 days. The animals were located at Manhattan, Kansas, and some were available for grub counts the following fall and winter of 1957–58.

July 17, 1957, 37 Hereford steers and heifers, averaging 527 pounds, received the first medicated protein supplement feed, in pellet form. It was fed at 4 lbs./head/day so that each averaged 2 mg. of ronnel/kg./day. The following 79 days the animals were fed the same ration except when rain made it impossible to reach the pasture. The feed containing the ronnel used the first 35 days was formulated 2 days prior to the first feeding. The second batch of feed was similarly formulated 35 days later, except that more ronnel was added to correlate the dosage with the weight gain of the cattle. Thirty-one untreated animals in another but comparable pasture served as controls.

The untreated animals were sprayed with lindane for biting flies on September 9, 1957.

Results.—Table 2, Experiment 2, shows virtually no reduction in grubs. Six of the 28 untreated animals and 3 of the 25 treated animals were infested.

EXPERIMENT 3.—This test was undertaken to compare the efficacy of early treatment with ronnel with later treatments used in Kansas in 1956 (Knapp *et al.* 1958). Purebred Hereford bulls and heifers owned by the C. K. Ranch, Brookville, Kansas, were used. They were kept in a barn during the heel fly season.

August 14, 1957, 22 show animals were treated with ronnel. Twelve received the newer type 15-gm. boluses, the other 10 the older 10-gm. boluses which had been stored for 1 year at room temperature. Eighteen comparable animals were left untreated. Grub counts were made only through February 1958.

Results.—Table 2, Experiment 3, based on the total number of grubs recorded throughout the three counts, shows that the treated animals had a 63.1% reduction in grubs. Twelve of the 13 untreated animals and 9 of the 13 treated animals were infested. No difference in grub reduction occurred between the two boluses used.

EXPERIMENT 4.—This test was conducted on the Stanley Marr farm, Jewell County, Kansas, to compare the effectiveness of a late treatment with ronnel with earlier treatments against cattle grubs.

November 11, 1957, 30 head of purebred Angus cows and heifers, nearly equally distributed within both groups, were bolused with ronnel. The animals' weights were estimated by the owner before treating. Ten similar animals were left untreated. No grubs were in their backs at the time of treatment. Additional grub counts were made in January and March 1958. No individual charts were made and all grubs found on the third count were regarded as new grubs.

Results.—Table 2, Experiment 4, shows that the treated animals underwent a 92% reduction of grubs, based on the total grubs found in the three counts made. Seven of the 30 treated animals and 8 of the 10 untreated were infested.

EXPERIMENT 5.—This test was designed to compare the efficacy of Dowco 109 as a suspension spray, an emulsion spray, and as a feed additive. The animals were

Table 2.—Results of ronnel, Dowco 109 and Co-Ral for cattle grub control in Kansas cattle.

Experiment No.	Treatment	Date 1957		No. Animals	Average Number of Grubs/ Animal	No. Animals Infested	Grub Reduction (%)
2	Ronnel, fed @ 2 mg./ kg./day for 79 days	[a]	Untreated	28	0.31	6	—
			Treated	25	0.35	3	0
3	Ronnel, bolus 110 mg./kg.	Aug. 14	Untreated	13	10.3	12	—
			Treated	13	3.8	9	63.1
4	Ronnel, bolus 110 mg./kg.	Nov. 11	Untreated	10	6.60	8	—
			Treated	30	0.53	7	92.0
6	Dowco 109, bolus 25 mg./kg.	Sept. 27	Untreated	12	2.8	5	—
			Treated	12	0	0	100.0
9	Co-Ral, spray 0.5%, 2 qt./head	Sept. 9	Untreated	29	0.31	6	—
			Treated	26[b]	0.23	1	25.8
				33[c]	0.30	7	3.2

[a] Low level feeding commenced July 17, 1957.
[b] Sprayed on back and sides only, lot 1.
[c] Complete coverage, lot 2.

Table 3.—Results of Dowco 109 for cattle grub control on registered Hereford bulls.

TREATMENT	DATE 1957	CONCENTRATION	RATE	NO. ANIMALS	AVERAGE NUMBER OF GRUBS/ANIMAL	NO. ANIMALS INFESTED	GRUB REDUCTION (%)
W.P.	Sept. 17	0.5%	1 gal./head	14	6.71	8	0
E.C.	Sept. 17	0.5%	1 gal./head	13	1.5	2	49.2
Feed	Sept. 23	15 mg./kg.	1 day feed	18	1.6	7	45.8
Untreated				20	2.95	1	

registered Hereford bulls owned by the C. K. Ranch of Brookville, Kansas, and were corralled at the JoMar Ranch east of Salina, Kansas, during the heel fly season.

September 17, 1957, 22 bulls were sprayed with a 0.5% Dowco 109 suspension at approximately 1 gallon per head, while milling around in a small pen. Another lot of 22 bulls was sprayed with a 0.5% Dowco 109 emulsion at the same rate as the suspension. Only three or four bulls of the latter group were sprayed at one time, thus getting a much better coverage than those that received the suspension.

September 23, 1957, 21 bulls were fed 15 mg./kg. of Dowco 109 in their ration, divided between two feedings, morning and evening. The animals were on full feed. This medicated feed was prepared by hand mixing the Dowco 109 concentrate with the daily ration. Twenty comparable bulls served as the untreated controls. No grubs were found in the backs at the time of treatment. One grub count was made in December 1957, and one in January 1958.

Results.—Table 3, based on the total number of grubs found in both counts, shows that the bulls treated with the 0.5% Dowco 109 wettable powder with poor coverage had no reduction of grubs compared with 49.2% reduction for the 0.5% emulsion, and 45.8% reduction with the Dowco 109 in the feed.

EXPERIMENT 6.—Twelve purebred Angus calves, consisting of bulls and heifers, and belonging to the University Department of Animal Husbandry, were treated with a bolus form of Dowco 109 to test the effectiveness of this material. Twelve were used as untreated controls. All were on pasture during the heel fly season except six, which were kept in a barn. The average individual weight was estimated to be 250 pounds.

September 27, 1957, nine were selected by gate cut and bolused with 25 mg. of Dowco 109/kg. Nine were left untreated. Three of the six calves kept in the barn were also treated and the remaining three left untreated. No grubs were found in their backs at the time of treatment. Grub counts were made monthly through February 1958.

Results.—The treated calves underwent a 100% grub reduction (Table 2, Experiment 6). Five of the 12 untreated calves were infested.

EXPERIMENT 7.—Bolus and spraying treatments were conducted at the Crofoot Feed Lot, Strong City, Kansas, November 11, and feeding commenced December 5, 1957. This experiment was designed to determine results of three materials under actual field conditions.

Grade feeder cattle which had spent the previous heel fly season in the Southern and Southwestern United States, were on full feed and weighed an average of 800 pounds when treated.

Dowco 109 and Co-Ral were sprayed by an inexperienced man hired by the cooperator. Only the necessary instructions were given, *i.e.*, per cent concentration, pounds pressure per square inch, to wet each animal thoroughly, and safety precautions were provided.

The Dowco 109 bolusing and dimethoate bolusing were done by the senior author, and the Dowco 109 fed over a 3-day period by the cooperator. Grub counts were made by the writers.

Twenty animals from a lot of 102 were selected by gate cut and bolused with 16.5 mg. of Dowco 109/kg. Another lot of 45 animals was bolused with 10 mg. of dimethoate/kg. Another lot of 37 animals was left untreated. Animals were marked according to treatment and returned to the same lot.

Eighty-three from another lot were selected by gate cut and sprayed with a 0.5% Dowco 109 emulsion. Another lot of 163 head was sprayed with a 0.5% Co-Ral suspension. Forty-six head from the former lot served as untreated controls. The animals were sprayed as they were driven back and forth in a large pen (ca. 60′×90′) past the spray operator.

After the spray operator had mixed each spray formulation, it was observed that he had no way of measuring the amount of water needed. In each case there appeared to be an excess of approximately 100 gallons of water above the amount needed or the specified concentrations. The same excess of spray was estimated to have been left in the sprayer after each treatment, indicating that the animals did not receive the amount of insecticide intended.

Spraying was done on a cool, windy, and damp day. In both the Co-Ral and Dowco 109 spray treatments most of the animals received a fair coverage, a few received a poor coverage, and one or two received but little coverage.

December 5, 1957, 166 animals were fed 4.5 mg./day of Dowco 109 in 1 pound of protein per day with their ration for 3 consecutive days. A Dowco 109 10% pre-mix was mixed the day previously with a 42% protein meal and then shipped to the Crofoot feed lot, where it was mechanically mixed with the regular ration.

Grub counts were made December 19, 1957, and January 23, 1958.

Results.—On the first count, 38 days after treatment, the animals treated with the Dowco 109 bolus showed a 93.7% reduction in grubs (table 4). Five of the 20 treated animals and 28 of the 34 untreated animals were infested. On second count, 73 days after treatment, the reduction was 96.5%. Three of the 18 treated animals at this count were infested, compared with 25 of the 37 untreated. Dowco 109 emulsion produced a 61.2% grub reduction at

Table 4.—Field results with Dowco 109, Co-Ral, and dimethoate for cattle grub control on grade feeder cattle at Strong City, Kansas.

Treatment Nov. 11, 1957	Formulation	Rate and Concentration	Counts After Treatment							
			38 Days (December 19, 1957)				73 Days (January 23, 1958)			
			No. Animals	Average Number of Grubs/ Animal	No. Animals Infested	Grub Reduction (%)	No. Animals	Average Number of Grubs/ Animal	No. Animals Infested	Grub Reduction (%)
Dowco 109	Bolus	16.5 mg./kg.	20	0.75	5	93.7	18	0.22	3	96.5
Dimethoate	Bolus	10 mg./kg.	40	3.4	27	71.4	42	4.3	14	30.6
Untreated	—	—	34	11.9	28	—	37	6.2	25	—
Dowco 109[a]	E	1 gal./head (0.5%)	81	12.1	63	61.2	83	6.9	71	27.4
Co-Ral[b]	W.P.	1 gal./head (0.5%)	163	2.8	84	91.0	157	4.2	84	55.8
Dowco 109[c]	Feed	5 mg./kg.[d]	155	4.8	109	84.6	166	1.6[e]	70	83.2
Untreated	—	—	46	31.2	46	—	40	9.5	38	—

[a] Emulsion spray applied at 300 p.s.i.
[b] Wettable powder spray applied at 300 p.s.i.
[c] Treated December 5, 1957.
[d] Per day for 3 days.
[e] Four animals had 40.7% of all grubs.

the first count after treatment. All the 46 untreated animals were infested compared with 63 of the 81 treated. Grub reduction on the second count was 27.4%. Seventy-one of the 83 treated animals were infested, compared with 38 of the 40 untreated.

Data given in table 4 also show that the Co-Ral-treated animals had a 91% reduction in grubs on the first count. Eighty-four of the 163 treated animals counted were infested, whereas all the untreated were infested. On the second count grub reduction was 55.8%. The number of treated animals infested remained the same in the second count (84 of 157 counted), while the untreated animals decreased (38 of 40 counted).

In the dimethoate experiment the treated animals showed a 71.4% reduction in grubs on the first count. Twenty-seven of 40 treated animals were infested, compared with 28 of the 34 untreated animals. On the second count the grub reduction was 30.6%. The number of animals infested decreased (12 of 37 untreated compared with 14 of 42 treated).

Data given in table 4 also show that animals fed Dowco 109 December 12, 1957, underwent 84.6% reduction in grubs 2 weeks after treatment. All the 46 untreated animals were infested, compared with 109 of the 155 treated animals. On the second count, 49 days after treatment, grub reduction decreased from 84.6% to 83.2%. Seventy of the 166 treated animals were infested, compared with 38 of the 40 untreated animals.

Experiment 8.—This experiment was conducted with native Hereford calves averaging 483 pounds at the Gooch Feed Mills, Juniata Ranch, Manhattan, to compare the efficacy of feeding Dowco 109 for 1, 6, and 12 days for cattle-grub control.

November 12, 1957, 10% active Dowco 109 in wheat bran was formulated with a 42% protein meal. Feeding was started November 20, 1957, at such a rate that the first lot of 18 calves received 15 mg. of Dowco 109/kg. for 1 day. The second lot of 21 calves received 2 mg. of Dowco 109/kg./day for 6 days. The third lot of 17 calves received 1 mg. of Dowco 109/kg./day for 12 days. Thus the calves received a total of 15, 12, and 12 mg. of Dowco 109/kg., respectively. Eighteen calves were left untreated.

Results.—Data in table 5 show that animals that received 15 mg./kg. for 1 day underwent a 73.1% reduction of grubs, while there was a 76.7% reduction at 2 mg./kg./day for 6 days, and 43.5% reduction at 1 mg./kg./day for 12 days. The number of calves infested with grubs were 9, 11, and 10, respectively, compared with 14 in the untreated.

Experiment 9.—September 9, 1957, at Manhattan, two lots of native Hereford steers and heifers averaging 600 pounds, were sprayed with a 0.5% Co-Ral suspension at the rate of 2 quarts per head to test the effectiveness of this formulation against cattle grubs. These animals were on pasture during the heel fly season and during the sprayings.

Twenty-six animals, lot 1, were run through a narrow chute and sprayed, being thoroughly wetted on the backs and sides only. Thirty-three animals in another pasture, lot 2, were thoroughly sprayed on the back, sides, and underside. The spraying of the underside was done by driving the animals around in a small pen and spraying upward from the ground.

Table 5.—Results with Dowco 109 fed to Hereford steer and heifer calves for three periods of consecutive days for cattle grub control.[a]

No. of Days	Total Dowco 109 Fed	No. Animals	Average Number of Grubs/ Animal	No. Animals Infested	Grub Reduction (%)
1	15 mg./kg.	18	5.2	9	73.1
6	12 mg./kg.	21	4.5	11	76.7
12	12 mg./kg.	17	10.9	10	43.5
	Untreated	18	19.3	14	

[a] Feeding started November 20, 1957.

Another lot of 29 animals, in a third pasture, was left untreated as controls. These animals were sprayed the same day with lindane for the control of biting flies.

The weather on the day of spraying was sunny and warm with no wind. A slight rain started 17 hours after spraying and continued intermittently the following day. September 11, a continuous rain fell, ending with 2.21 inches for the 24-hour period after treatment.

Results.—Table 2, Experiment 9, shows that only 6 of 29 untreated animals were infested. In lot 1, 1 of 26 animals was infested with a 25.8% average reduction in grubs. Lot 2 had 7 of 33 infested, with a 3.2% reduction.

DISCUSSION.—Results of early treatment with ronnel indicated that in parts of Kansas where experiments had been conducted, native cattle could be treated effectively for cattle grubs with certain systemic insecticides as early as June. August treatments with ronnel indicated insufficient grub reduction. Wade & Colby (1958), treating different groups of Texas cattle at monthly intervals from May to November with ronnel, found that the average number of grubs per animal in the backs was greatly reduced in all treatments except those of August. Burns & Goodwin (1958), however, received good control on southwest Louisiana cattle with both ronnel and Co-Ral in August treatments but less effective control in June treatments, although in another experiment they received good control in June and July with both compounds. Harris *et al.* (1959) found Co-Ral to be more effective than an oral drench of ronnel when applied in the Florida Everglades in May, June, July, or August. A June application of ronnel was less effective than those made in May through September.

Gains in weight obtained following a ronnel treatment in June, July, or August indicated that if treated early, animals may not have a setback in weight as indicated when treated in late fall or early winter (Brethour *et al.* 1958, 1959). No explanation has been given as to why the July treatment caused a weight reduction and the June and August treatments did not. Jones & Matsushima (1959) found that weanling calves treated with ronnel boluses in December 1957 gained more than the untreated when grubs were more numerous in the backs. During the early and latter parts of the 221-day feeding experiment, however, the untreated outgained the treated. These differences, they report, were not significant. Neel (1958) also reported no significant difference in weight gain following treatment with systemics. The senior author (unpublished data) has found that any initial weight loss on yearling steers, resulting from systemic treatments, will be regained before market time 1 year later. Brethour *et al.* (1959) found that weight loss caused by late-season treatment was due possibly to the number of grubs killed in the animals' back from the systemic insecticide.

Because of low grub infestation, no conclusions can be drawn from the studies in which low-level feeding of ronnel was evaluated. However, fewer treated animals were infested, compared with the untreated. In an experiment reported by Sturdy (1958) effective control of grubs was achieved when ronnel was incorporated in a protein supplement and fed continuously at different levels for 8 months. Wientraub *et al.* (1958) and Rogoff & Kohler (1959), both using ronnel incorporated in a mineral mixture and given to calves on a free-choice basis over a number of days (collectively 28 to 93 days), reported effective grub control. In another low-level feeding test by Howell *et al.* (1958), ronnel was incorporated in a concentrated ration and fed at the rate of 5 mg./kg./day for 25 days, and 10 mg./kg./day for 12 days, with effective control. French *et al.* (1958) also found ronnel to be effective when fed at the rate of 35 mg./kg./day for 6 days. The senior author (unpublished data), however, has found that ronnel at 8 mg./kg. fed daily to 4-year-old Hereford beef cows in alfalfa meal for 14 consecutive days gave no appreciable grub control. This fact indicates a possibility that a certain minimum dosage is required for grub control and/or that ronnel may not be compatible with certain feeds, breaking down before consumption.

Results of Dowco 109 used as a feed additive indicates that 5 mg./kg./day for 3 days is promising and warrants further study. The longer, low-level feeding indicates that 6 days at 2 mg./kg./day is more effective than 12 days at 1 mg./kg./day, but indications are that neither gave satisfactory control at these levels. Fifteen mg./kg. as a 1-day feed additive gave results similar to the 6-day feeding in one experiment, and similar to the 0.5% emulsifiable concentrate in another. None of the Dowco 109 feed experiments gave results comparable with the 0.75% spray, or bolus treatment, as reported by Brethour *et al.* (1959) and Roth & Eddy (1959), nor did it compare with the 1-day feeding of ronnel reported by Knapp *et al.* (1958). However, DeFoliart *et al.* (1958), French *et al* (1958), and Howell *et al.* (1958) all reported effective control with Dowco 109 when mixed with certain feeds and fed 1, 2, or 10 days; 6 days; and 1 or 3 days, respectively. The total dosage used in all trials was approximately 15 mg./kg. except that the 10-day feed totaled 20 mg./kg.

The late-season spray treatment of Co-Ral and Dowco 109 applied by an inexperienced spray operator at a concentration of 0.5% greatly reduced the number of grubs on the first count after treatment with Co-Ral but not with Dowco 109. Records were not kept as to whether all the larvae in the backs of the treated animals were killed and the remaining live ones were new larvae that had migrated to the back during the period between the two counts. Late-season treatment may have been responsible for the low reduction of grubs with Co-Ral on the second count instead of poor coverage or a weak concentration of spray, since Brethour *et al.* (1958) reported good control with Co-Ral with a top-line spray of 0.34%, and Burns & Goodwin (1958) reported good control at 0.25%. Since Dowco 109 as a 0.5% spray gave consistently poor control of grubs in all experiments, it cannot be stated that the poor control by the late-season treatment of Dowco 109 was actually the result of a low-spray concentration, although the low concentration could have been a contributing factor.

Dimethoate, used as a late-season treatment at the rate of 10 mg./kg., followed the same general trend as the Co-Ral late-season treatments, *i.e.*, a fairly good control was obtained during the first few weeks after treatment, indicating that the grubs in the back at the time of treatment were killed, but migrating grubs were apparently not killed, since numerous grubs were found during the second count. Data presented by Howell *et al.* (1958) confirm these observations.

In no test reported here was there a sign of toxicity in any animal treated.

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Pollination of Red Clover in Minnesota[1]

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ABSTRACT

Observations on pollination of mammoth red clover (*Trifolium pratense* L.) in Roseau County of northern Minnesota indicated that honey bees (*Apis mellifera* L.) remained at a low level of abundance on red clover until sweetclover (*Mellilotus alba* Desr.) had gone out of bloom. Late-season honey bee activity on mammoth red clover was important in effecting late seed crops of 138 to 354 pounds of seed per acre. In Clearwater and Polk Counties of northwestern Minnesota, where medium red clover was the only legume grown for seed, honey bees were important pollinators of the medium red clover. Partial correlation calculated from observations on 18 fields of second-crop medium red clover indicated that honey bees were more *important* than bumble bees (*Bombus* spp.) in determining seed yields. Partial regressions of yields on average counts of honey bees and bumble bees indicated that bumble bees were more *efficient* than honey bees. Abundance of honey bees and seed yields decreased as the distance between red clover fields and apiaries increased. Pollination by honey bees accounted for yields of 193 to 456 pounds per acre when bees were located adjacent to the fields of medium red clover, while at distances of greater than 1 mile from the nearest apiary pollination by honey bees was insignificant.

Seed production of red clover, *Trifolium pratense* L., provides an important supplementary income for many farmers in Minnesota. The acreage of red clover harvested for seed each year varies from approximately 40,000 to 80,000 acres. Although a considerable number of farmers grow red clover primarily for seed, a common practice is to grow the red clover for the first cutting of hay. If hay is in good supply, the second crop of red clover is left for seed. Frequently no arrangements are made for control of injurious insects or for pollination of the seed crop. This fact probably accounts, at least in part, for the low average yields of 58 to 75 pounds per acre that have been reported for the State during recent years.

IMPORTANCE OF BUMBLE BEES VS. HONEY BEES IN POLLINATION.—Observations in red clover fields of Minnesota during the past 9 years indicate that lack of pollination is the most critical factor resulting in unsatisfactory seed yields. Experiments on the control of injurious insects have demonstrated that a single bud-

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stage application of insecticide often results in a moderate increase in seed yield; however, seed yields of red clover in northern Minnesota have responded less to the use of insecticides than have yields of either alfalfa (*Medicago sativa* L.) or alsike clover (*Trifolium hybridum* L.). Therefore, pollination has received a major portion of our attention. Field experiments have been conducted since 1953 in an attempt to determine the extent to which honey bees (*Apis mellifera* L.) could be used to pollinate red clover under Minnesota conditions. Furthermore, we have been interested in the relationship between the abundance of honey bees and bumble bees (*Bombus* spp.) and seed yields. Such information can be of considerable aid to a grower in planning a pollination program.

Many workers have reported during the past 45 years that honey bees are important pollinators of red clover under certain conditions (Westgate & Coe 1915, Richmond 1932, Stapel & Erickson 1936, Dunham 1939, 1943, Anderson & Wood 1944). Woodrow (1952) described in detail the tripping of red clover florets by honey bees. The fact that corolla tubes in red clover are longer than the tongues of honey bees has been offered as an explanation for the relative unattractiveness of red clover for honey bees. However, convincing evidence has been presented that no relationship exists between lengths of corolla tubes in red clover and effectiveness of honey bees in pollinating the crop (Richmond 1932, Ballantyne 1938, Wilsie & Gilbert 1940, Armstrong & Jamieson 1940, 1941, and Starling *et al.* 1950). The nectar in the red clover florets frequently rises to a height where honey bees can reach it, and they do collect nectar even if they are not able to obtain all the nectar from each floret. Honey bees may collect pollen or both pollen and nectar on the same foraging trip.

Greater attractiveness of other crop plants to bees is a critical factor in pollination of red clover by honey bees. The importance of sweetclover has been emphasized by Westgate & Coe (1915), Stapel & Erickson (1936), Dunham (1939), Anderson (1948), and others.

Close relationships between abundance of honey bees on red clover and seed yields have been reported by Haydak (1938), Dunham (1943, 1951), Walstrom *et al.* (1951), and Butler *et al.* (1956). Most of these were general observations and not expressed quantitatively, although Dunham (1951) did state that one honey bee per square yard could result in a yield as high as 12 bushels of seed per acre.

It has similarly been a common observation that abundance of honey bees and seed yields of red clover decrease with an increase in distance from nearby apiaries (Haydak 1938, Alpatov 1948, Braun *et al.* 1952, 1953). Honey bees have been reported to decrease rather sharply from as close as 400 to 600 feet from the apiary (Walstrom 1958) to a distance of $\frac{3}{4}$ mile (MacVicar *et al.* 1952) or even 1 mile (Haydak 1938).

Bumble bees are generally recognized to be more efficient pollinators than honey bees, because they visit more florets per unit of time, work longer hours, and work under more adverse weather conditions (Westgate *et al.* 1915, Gubin & Romashoff 1933, Dunham 1939, Bird 1944, Starling *et al.* 1950). Schwan (1953) expresses this relationship quantitatively as 1 bumble bee being equal to 2.5 honey bees in efficiency. In spite of the greater efficiency of bumble bees, they are not sufficiently abundant for adequate pollination, and honey bees are responsible for a large proportion of the seed crop (Dunham 1939, 1943). Braun *et al.* (1953) reported a ratio of 2.2 honey bees per bumble bee on red clover when 2 colonies of honey bees per acre were located near the field.

METHODS.—Three fields of mammoth red clover were selected in 1955 in each of two areas of Roseau County in northern Minnesota for observations on the abundance of honey bees on red clover as influenced by the presence of alsike clover and sweetclover (*Mellitotus alba* Desr. and *M. officinalis* (L.) Lam.). All these legumes are commonly grown for seed in this area. Selection of the two observation areas, one near Roseau and one near Salol, Minnesota, was based on the presence of one or more apiaries in each area with several fields of mammoth red clover within flight range. All acreages of red clover, alsike clover, yellow sweetclover, white sweetclover, and alfalfa within a 2-mile radius were located and mapped in each area. The method of estimating pollinators was somewhat similar to that used by Vansell & Todd (1946). Ten $6' \times 6'$ plots were staked out in each field selected for observation. Just prior to staking, each red clover field was sprayed with $1\frac{1}{2}$ lbs. actual DDT per acre during the bud stage to control plant bugs. Pollination plots were located every 20 to 40 paces along a curved path through each field. Plots were located in one or more fields of white sweetclover, yellow sweetclover alsike clover, and alfalfa in addition to the three fields of red clover in each area. Honey bees and bumble bees were counted periodically in each of the field plots for 1-minute periods. The red clover plots were harvested by hand. The samples were dried in cloth flour sacks and threshed individually in a special threshing machine.

A second series of observations was conducted on 18 fields of medium red clover in Clearwater and Polk Counties of northwestern Minnesota where alsike clover and sweetclover are scarce. Minnesota common medium red clover is grown in this area. One strain is known locally as Swedish medium red clover. Growers usually cut the first crop for hay between June 20 and July 1 and leave the second crop for seed. Observations were conducted on a total of 18 fields during the period from 1953 to 1956. The fields were selected according to distance from apiaries. This area was ideal for this type of observation because apiaries were widely scattered and opportunity was afforded to observe the effects of distance from the apiary on honey bee populations and seed production. Following an application of DDT to control injurious insects, ten $6' \times 6'$ plots were staked out in each red clover field. Plots were located randomly along a curved path through each field. Four $2' \times 3'$ screen cages were placed in each of three fields in order to determine seed yields when bees were excluded. Honey bees and bumble bees were counted in the field plots for 1-minute intervals periodically during the flowering period for a total of 15 to 20 counts in each plot. The plots were harvested by hand, and samples were threshed individually for estimates of seed yields.

POLLINATION OF MAMMOTH RED CLOVER IN RELATION TO THE PRESENCE OF OTHER CLOVERS.—The observed blossoming periods of legume crops in the Salol area and results of honey bee counts are illustrated in figure 1. Alsike clover came into bloom early in the season and

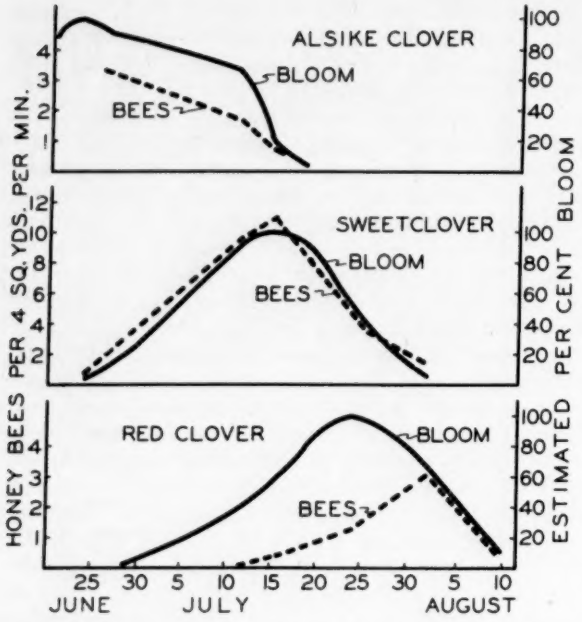

FIG. 1.—Honey bee counts in relation to blossoming periods of clovers. Salol, Minnesota. 1955.

reached a peak about June 22. Only the last 2 weeks of alsike bloom overlapped the blossoming period of mammoth red clover, and alsike clover did not appear to attract many bees from the red clover. White sweetclover reached maximum bloom in mid-July about the time that alsike went out of bloom and about 10 days before the red clover reached its peak of bloom. The abundance of honey bees on alsike and sweetclover was closely related to the amount of bloom. However, honey bee activity on red clover did not reach a peak until the sweetclover had gone out of bloom.

Blossoming periods of legume crops and results of honey bee counts in the Roseau area are illustrated in figure 2. Here, as in the Salol area, peaks of bee activity were associated with peaks of bloom in alsike clover, yellow sweetclover, and white sweetclover. A high second peak of bee activity occurred on white sweetclover during the latter part of the blossoming period apparently as a result of cessation of bloom in the alsike and yellow sweetclover. Honey bees remained relatively scarce on red clover until the sweetclover went out of bloom.

The late-season bee activity on red clover resulted in a late seed crop with yields ranging from 138 to 324 pounds per acre. The red clover fields nearest the apiaries tended to have the most honey bees and the highest seed yields (table 1). The major exception was Field A. This field was located on a light sandy soil, and the red clover went out of bloom while the honey bees were still working sweetclover.

Bumble bees were scarce in the Roseau observation area during 1955 (table 1), and they contributed little to pollination of red clover. In the Salol area, bumble bees were somewhat more numerous. *Bombus borealis* Kirby was the principal species followed by *B. terricola* Kirby, *B. vagans* F. Smith, and *B. rufocinctus* Cress.

Pollination studies on first-crop medium red clover in

Table 1.—Relation of honey bee abundance and seed yields of mammoth red clover to distance from the nearest apiary. Roseau and Salol, Minnesota, 1955.

Field	Distance from Apiary (miles)	Average Bees/4 Sq. Yds./Min.		Yield (lb./A.)
		Honey Bees	Bumble Bees	
Roseau area				
A	0.12	0.43	0.02	138
B	0.25	1.00	0.05	354
C	0.5	0.37	0.08	156
Salol area				
D	0.38	0.73	0.34	324
E	0.88	0.42	0.18	259
F	0.88	0.15	0.29	245

Roseau County during 1956 and 1957 indicated that the blossoming period of medium red clover coincided with that of alsike and sweetclover. As a result, honey bee populations remained low on medium red clover throughout most of its blossoming period, and seed yields were low. The growing season is too short in this area to permit the practice of harvesting the first crop for hay and growing the second crop for seed. However, an early clipping to delay the flowering period of medium red clover might be helpful.

POLLINATION OF SECOND-CROP MEDIUM RED CLOVER.—In the red clover seed producing area of Clearwater and eastern Polk Counties, honey bees were consistently more numerous on red clover than in Roseau County in spite

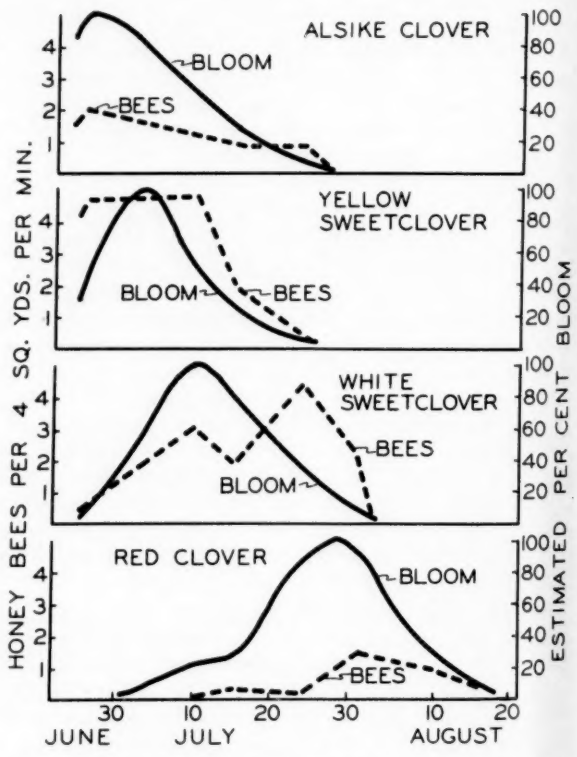

FIG. 2.—Honey bee counts in relation to blossoming periods of clovers. Roseau, Minnesota. 1955.

Table 2.—Relationships of honey bee abundance and seed yields to the distances between fields of second crop medium red clover and the nearest apiary. Clearwater and Polk Counties, Minnesota.

Distance from Apiary (miles)	Average Bees/4 Sq. Yds./Min.		Yield (lb./A.)	Yield Owing to Honey Bees
	Honey Bees	Bumble Bees		
1953—0	2.16	0.30	540	456
1.0	0.67	0.24	190	129
1954—0	3.01	0.43	507	426
0.75	1.85	0.62	469	324
1.25	0.56	0.34	116	64
3.00	0.04	0.99	383	11
1955—0	1.30	0.07	222	207
0.25	1.71	0.09	224	209
1.00[a]	1.78	0.09	271	254
1.00	0.43	0.78	270	79
1.75	0.02	0.05	32	7
4.00	0.02	0.09	42	6
1956—0	1.73	0.56	276	193
0.25	1.60	0.56	370	252
1.50[b]	0.97	1.35	235	82
1956—0.25	1.27	0.30	162	123
0.50	1.90	0.35	247	198
1.25	0.25	0.33	46	17

[a] Equidistant from two apiaries.
[b] Honey bees quite numerous at one end of field as if from a bee tree, but the source was not found

of a lower concentration of apiaries in the former area. This appeared to be due primarily to the absence of sweetclover in the Clearwater-Polk area. The main flowering crop other than red clover was alfalfa, which had been allowed to come into bloom before it was cut for hay. Bumble bees were numerous in some fields of red clover, but they varied tremendously from one location to another.

Honey bees and bumble bees were the only important cross-pollinators of medium red clover. The close association between seed yields and abundance of honey bees and bumble bees is indicated by the highly significant multiple correlation of 0.818 (15 degrees of freedom). A higher partial correlation between honey bee counts and seed yields (0.79) than between bumble bee counts and seed yields (0.57) indicated that honey bees were more important than bumble bees in this area during the period of our observations. It is evident from the last two columns in table 2 that honey bees were responsible for a major portion of the seed yield in most fields. Bumble bees appeared to be of relatively greater importance than reported by Dunham (1939, 1951) for Ohio and of relatively less importance than reported by Bird (1944) for Quebec, Canada. A significant negative partial correlation (−0.49) between honey bees and bumble bees, with yields held constant, points to the possibility of some competition between the two groups. Certainly, there does not appear to be a high degree of competition, but bumble bees did tend to be somewhat more abundant in fields located more than 1 mile from apiaries.

Partial regressions of seed yields on the average counts of honey bees and bumble bees were calculated to evaluate the efficiency of each type of pollinator. Results are presented in figure 3. The regression equation for these data is:

$$Y = 26 + 132X_1 + 176X_2$$

where Y is the predicted yield in pounds per acre, X_1 is the average number of honey bees per 4-square-yard plot per minute, and X_2 is the average number of bumble bees per 4-square-yard plot per minute. Interestingly enough, the constant of 26 in the regression equation approximates the average yield of 23 pounds of seed per acre obtained in field cages from which bees were excluded. According to the regression formula, with an average of four honey bees per 4 square yards per minute, we might expect a yield of about 554 pounds of seed per acre. This supposition agrees reasonably well with Dunham's conclusion (Dunham 1951) that one honey bee per square yard may result in seed yields up to 720 pounds per acre. The ratio of the partial regressions indicated that bumble bees were 1.33 times as efficient as honey bees in pollinating medium red clover. We believe this to be a conservative estimate because unfavorable weather probably minimized the differences in efficiency. A drought in 1955 and cold wet weather in 1956 prevented the realization of potential red clover yields. Partial regressions of yields on honey bee and bumble bee counts for 89 individual plots in four fields during the favorable 1954 season indicated that 1.84 honey bees equaled 1 bumble bee in efficiency during that year. Even this ratio does not reach the 2.5:1 ratio reported by Schwan (1953). We prefer to accept the 1.33:1 ratio for our results because it is based on field averages

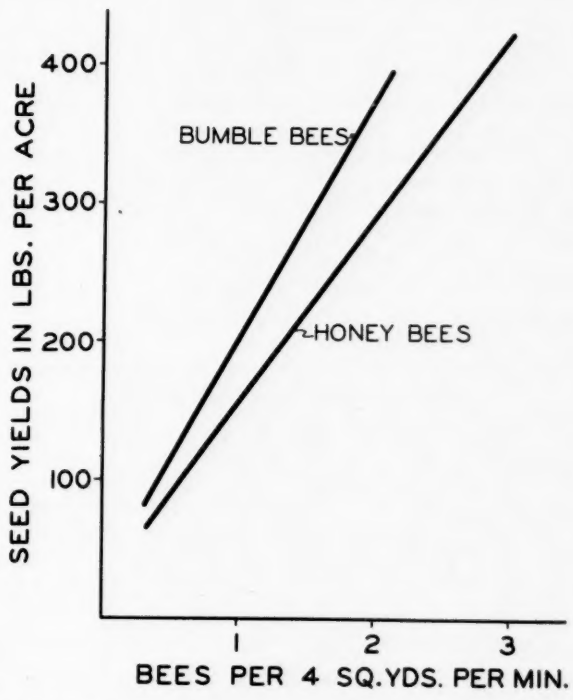

FIG. 3.—Partial regressions of honey bee counts on seed yields with bumble bees held constant and bumble bee counts on seed yields with honey bees held constant. Second crop medium red clover. Clearwater and Polk Counties, Minnesota.

over a 4-year period; however, we do believe that under some conditions bumble bees may demonstrate a relatively higher efficiency than this ratio indicates. The superior efficiency of bumble bees is substantiated by observations that bumble bees work longer hours in the field and visit more florets per minute than honey bees. Similar observations have been reported by Westgate & Coe (1915), Gubin & Romashoff (1933), and Dunham (1939).

The predominant species of bumble bee on red clover in the Clearwater–Polk area was *Bombus borealis*. The numbers of this species exceeded the total of all other species combined. A few *Bombus fervidus* (F.) occurred, but these were not numerous and were not separated from *B. borealis* in the field. *B. fervidus* tends to be a more southern species in Minnesota. Morrison (1943) reported it to be the most abundant species on red clover in Quebec. Small numbers of *B. vagans*, *B. ternarius* Say, *B. rufocinctus*, and *B. terricola* were also recorded on red clover. *B. terricola* has been observed to be an important pollinator of alfalfa in northern Minnesota, but it is relatively rare on red clover. This species was rather common in one field of medium red clover in 1955, where it was puncturing the bases of the red clover florets to obtain nectar. *Bombus rufocinctus* was also puncturing the bases of the florets in the same field. Schwan (1953) reported similar stealing of nectar by *Bombus terrestris* (L.) in Sweden.

The relation of honey bee abundance and seed yields in relation to distance of the red clover fields from an apiary is indicated in table 2. The last column in the table gives the calculated yield accountable to pollination by honey bees if 1.33 honey bees equal 1 bumble bee in efficiency. A marked decrease in abundance of honey bees was usually apparent beyond 0.5 mile from the nearest apiary, and honey bee counts dropped to a low level beyond a distance of 1 mile. The only red clover field to receive appreciable pollination by honey bees at a distance of 1 mile was midway between two apiaries that were 2-miles apart. It seems quite obvious that for most effective pollination of red clover by honey bees the apiary should be located in the field, bordering the field, or at least within one-fourth mile of the field.

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The Effectiveness of Chlorinated Hydrocarbon Insecticide Mixtures for Control of Resistant and Susceptible Boll Weevils[1]

J. R. Brazzel[2] and D. A. Lindquist[3]

Abstract

The toxicity of toxaphene and DDT alone and combined in various proportions on laboratory-reared toxaphene resistant and susceptible strains of the boll weevil was studied. Toxaphene-DDT mixtures exhibited synergistic effects on resistant weevils, but only additive effects for susceptible ones. A mixture of four parts toxaphene and one part DDT was the most effective combination tested against resistant weevils. A dosage of 5 to 15 micrograms of toxaphene in combination with DDT was required to produce 50% mortality of resistant weevils regardless of the amount of DDT in the combination.

No evidence of synergism was found using mixtures of toxaphene with methoxychlor or DDE. A mixture of Strobane-DDT was slightly less effective than toxaphene-DDT, but the synergistic effect was evident with resistant weevils. Strobane® is a mixture of chlorinated terphenes with about 66% of chlorine.

No evidence was found of seasonal tolerance in field-collected boll weevils to a 2-1 mixture of toxaphene-DDT.

Prior to 1955 economical control of the boll weevil, *Anthonomus grandis* Boh., was obtained with certain chlorinated hydrocarbon insecticides. Resistance to these insecticides was first reported in 1955 by Roussel & Clower and subsequently by Walker *et al.* (1956) and Fye *et al.* (1957). Generally, with the occurrence of resistance in an area, the growers used calcium arsenate or organophosphorus insecticides for weevil control. In some areas various mixtures of chlorinated hydrocarbons were applied for control of resistant weevils. Toxaphene plus DDT appeared to be the most promising of the mixtures and extensive field tests during the past 3 years (1957–59) have shown that resistant boll weevils may be controlled with this combination of insecticides.

DDT is considered to be ineffective for control of the boll weevil, Bondy & Rainwater (1946), Parencia *et al.* (1946). Toxaphene is an effective material for control of susceptible weevil populations but not for resistant ones. Gaines *et al.* (1951) reported increased effectiveness of a toxaphene-DDT mixture over toxaphene alone for control of susceptible boll weevils. Roussel *et al.* (1959) reported the toxaphene-DDT mixture exhibited synergistic effects against resistant weevils but additive effects for susceptible ones.

The phenomenon of two insecticides, ineffective when used separately for control of resistant weevils but effective in control when applied as a mixture, indicated a need for further research. The data reported herein are the results of tests designed to compare the effectiveness of various combinations of toxaphene and DDT on standard laboratory cultures of resistant and susceptible boll weevils and to determine the critical amount of the two insecticides necessary to obtain effective boll weevil control.

Methods.—All boll weevils used in this study were reared in the laboratory in petri plates on an artificial diet, except as indicated in table 3. The rearing procedure and diet used were described by Brazzel *et al.* (1959). All the weevils were progeny of weevils collected in Mexico in 1957. This collection was divided into two groups. One of the latter was not subjected to insecticides. This culture is the susceptible strain, College Station-susceptible (CS-S). The other culture was selected with endrin for 14 generations, and by the latter part of 1958 had developed a 50- to 75-fold increase in resistance to endrin when compared with the susceptible culture (Davich, 1958 unpublished data). The resistant strain used in this study had attained this level of resistance by September 1958, and was not subjected to further selection pressure in an attempt to maintain a constant level of resistance for the duration of the experiment, College Station-resistant (CS-R).

Weevils were collected at 2- or 3-day intervals from the rearing containers. The adults were fed for 2 to 3 days on potted seedlings in the cotyledonary stage. Weevils used in these tests ranged from 2 to 5 days in age and had access to food for 2 or 3 days.

Dosage-mortality data were obtained by application of one microliter of acetone containing the insecticides to the dorsal metathoracic region. The calibrated micrometer and syringe device used was similar to the type commonly used by workers for topical applications. Dosage-mortality curves were obtained by treating groups of weevils with five dilutions of each insecticide. The highest dose for each was 150 μg./weevil with each dilution one-half the concentration of the preceding one. In case the mortality was too high in the low dilution, lower doses were used and the higher ones omitted.

Weevils numbering 600 to 1,000 were used to obtain each dosage-mortality curve. Most tests consisted of treating groups of 20 weevils with each of the five doses and an acetone-treated check. Each test was repeated five times. Insofar as possible, comparisons between resistant and susceptible strains with each insecticide combination were made simultaneously.

Treated weevils were held in groups of 10 in half-pint jelly glasses with perforated lids. Cotton seedlings as described for feeding during the pretreatment period were furnished for food. The treated insects were held at 83° to 85° F. until mortality records were made at the end of 48 hours. The dosage-mortality curves and LD-50's were computed by probit analysis. All values of toxicant refer to total amount applied. For example, the LD-50 for toxaphene-DDT at a ratio of 2 to 1 against resistant

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Table 1.—Mortality of resistant and susceptible boll weevils treated topically with DDT or toxaphene.[a]

	Per Cent Mortality			
	Susceptible		Resistant	
μG./Weevil	DDT	Toxaphene	DDT	Toxaphene
150.0	34	87	31	31
75.0	24	88	21	22
37.5	28	82	18	8
18.8	35	65	17	9
9.4	33	46	34	7

[a] 160-175 weevils tested at each dosage.

weevils was 15 micrograms of toxicant per weevil, of which 10 micrograms were toxaphene and 5 micrograms were DDT.

Boll weevils were weighed in groups of 10 at the beginning of this study. Since the average weight was 14.5 mg./weevil with less than 1 milligram variation, the weighing was discontinued and the data are presented in this study as micrograms of toxicant per boll weevil.

The following chemicals were used:

p, p′-DDT: 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane
p, p′-DDE: 1,1-dichloro-2, 2-bis (*p*-chlorophenyl) ethylene
Methoxychlor: 1,1,1-trichloro-2, 2-bis (*p*-methoxyphenyl) ethane
Toxaphene: Technical, chlorinated camphene containing 67% to 69% of chlorine
Strobane®: A terpene polychlorinate with a chlorine content of approximately 66%.

Results and Discussion.—*Toxaphene-DDT.*—The dosage-mortality data for toxaphene and DDT alone against resistant and susceptible boll weevils are shown in table 1. DDT was ineffective and resulted in similar mortalities with both strains. The estimated LD-50 of toxaphene against susceptible weevils was 8.9 μg./weevil. Against resistant weevils, however, toxaphene was ineffective; the extrapolated LD-50 was 575 μg. of toxaphene/weevil. These results show that DDT was not effective against either strain and that a high level of resistance to toxaphene existed in the resistant strain.

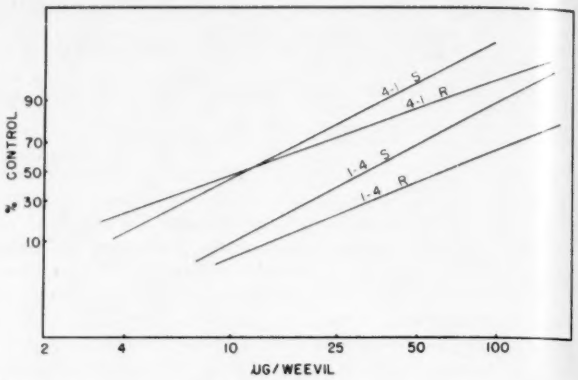

Fig. 2.—Dosage-mortality curves for resistant (R) and susceptible (S) boll weevils treated topically with toaxphene-DDT mixtures of 4–1 and 1–4.

The dosage-mortality curves for the toxaphene-DDT ratios of 1–2, 1–4, 1–10, 2–1, 4–1, and 10–1 are shown in figures 1, 2, and 3. The mixtures are identified by the ratio of toxaphene to DDT with the amount of toxaphene always given first; the μg./weevil refers to total toxicant applied. All combinations, except the 4 parts toxaphene to 1 part DDT, were significantly more toxic to susceptible weevils than to resistant ones. No outstanding differences in slopes of the dosage-mortality curves were noted. The increased effectiveness of the mixtures is shown by a shift of the curves with little change in slope. These data clearly indicate that the mixtures containing high proportions of toxaphene were more effective than those containing high proportions of DDT.

Figure 4 illustrates graphically the LD-50 values for toxaphene alone and in various combinations with DDT against resistant and susceptible boll weevils. The resistant strain is about 70 times as resistant to toxaphene as the susceptible strain; however, the resistant strain is only about two times as resistant to the toxaphene-DDT combinations, except the 4–1 mixture, as the susceptible strain. The 4–1 mixture of toxaphene-DDT was the most effective mixture against the resistant strain with an LD-50 of 11 μg./weevil. However, the LD-50's obtained

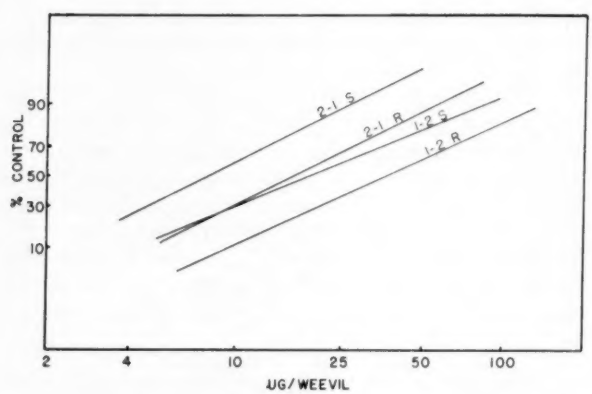

Fig. 1.—Dosage-mortality curves for resistant (R) and susceptible (S) boll weevils treated topically with toxaphene-DDT mixtures of 2–1 and 1–2.

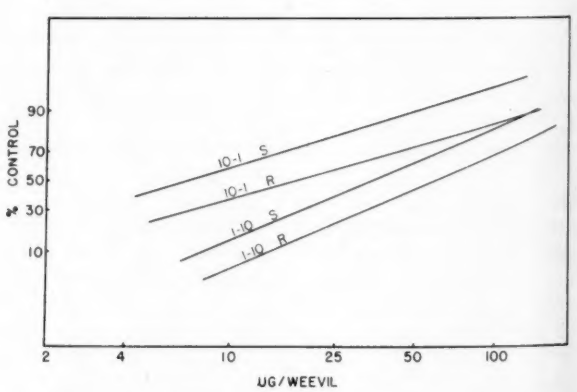

Fig. 3.—Dosage-mortality curves for resistant (R) and susceptible (S) boll weevils treated topically with toxaphene-DDT mixtures of 10–1 and 1–10.

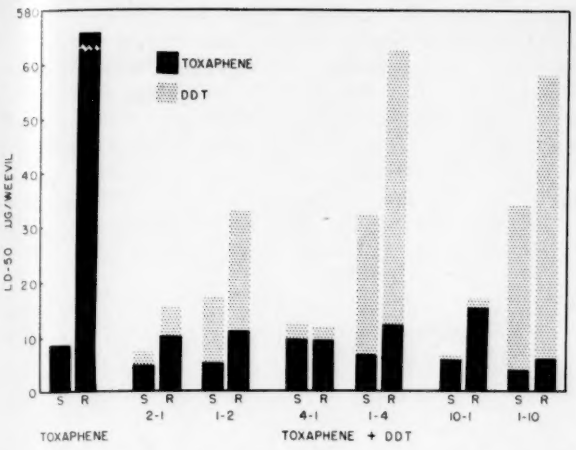

FIG. 4.—LD-50s of resistant (R) and susceptible (S) boll weevils treated topically with toxaphene and toxaphene-DDT mixtures at the ratios indicated. The relative proportion of each toxicant in a mixture is illustrated graphically.

for the 2–1 and 10–1 mixtures with the resistant strain were not significantly different from the 4–1 mixture.

The LD-50s for susceptible weevils treated with 2–1, 4–1, and 10–1 mixtures were similar to toxaphene alone. These data are in accord with the reports of Gaines *et al.* (1951) and Roussel *et al.* (1959) that toxaphene-DDT combinations show slightly additive effects but no evidence of synergistic action on susceptible weevils.

Synergistic activity of the toxaphene-DDT mixtures against resistant weevils is clearly indicated. Doses of 150 µg./weevil of toxaphene and DDT alone on resistant weevils resulted in mortalities of 31% and 31% (table 1), respectively. A mixture of 4 parts toxaphene and 1 part DDT killed 50% of this strain of weevils at a total dosage of 11 µg./weevil (fig. 4).

LD-50 values ranging from 32 to 60 µg./weevil were obtained with the 1–2 mixture on resistant weevils and the 1–4 and 1–10 mixtures on both strains. The LD-50s for the 2–1, 4–1, and 10–1 mixtures ranged from 6.3 to 17 µg./weevil for both strains. These results indicate that when DDT is present in greater amounts than toxaphene, the efficiency of the mixture is reduced.

Four to 15 micrograms of toxaphene per weevil were necessary in the mixture to kill 50% of the boll weevils, regardless of the amount of DDT present. In mixtures that contained more DDT than toxaphene the excess DDT apparently acted as a diluent. The data obtained with the 10–1 mixture on the resistant strain indicate that the ratio of DDT to toxaphene was too low and in the 2–1 mixture there was an excess of DDT. These results show that a relatively small amount of DDT in the mixture was sufficient to produce the synergistic effect.

Toxaphene-DDE and Toxaphene-Methoxychlor Mixtures.—The results of tests with toxaphene-DDE (2–1) and toxaphene-methoxychlor (2–1) are given in table 2. A dose of 150 µg. of DDE/weevil was nontoxic to resistant boll weevils. Toxaphene-DDE in a 2–1 ratio at a dose of 150 µg./weevil total toxicant resulted in only 47% mortality. These results indicate the addition of DDE to toxaphene had little effect on the effectiveness of toxaphene against resistant weevils.

Table 2.—Per cent mortality of resistant boll weevils treated topically with toxaphene, DDE, methoxychlor, toxaphene-DDE (2–1), and toxaphene-methoxychlor (2–1).

µG./ WEEVIL	TOXAPHENE[a]	DDE[b]	METHOXYCHLOR[b]	TOXAPHENE-DDE[b]	TOXAPHENE-METHOXYCHLOR[b]
150.0	31	0	38	47	76
75.0	22	—	22	24	53
37.5	8	—	0	16	32
18.8	9	—	3	1	26
9.4	7	—	1	6	5

[a] 160 weevils tested at each dosage.
[b] 60–95 weevils tested at each dosage.

Toxaphene or methoxychlor applied alone at 150 µg./weevil resulted in 31% and 38% mortality, respectively. A 2–1 mixture of the two insecticides at the same dose gave 76% mortality of the resistant weevils. These results indicate slightly more than additive effects of the two insecticides alone, but no definite synergistic action as was exhibited by the toxaphene-DDT mixtures.

Strobane-DDT.—Dosage-mortality curves for Strobane on susceptible weevils and Strobane-DDT mixture in a ratio of 2–1 on resistant and susceptible strains are given in figure 5. A dose of 150 µg. of Strobane/weevil on the resistant strain produced only 20% mortality and it was not possible to obtain a dosage-mortality curve. The curve for the 2–1 mixture on resistant weevils was almost identical with the curve for Strobane alone on susceptible weevils. This fact clearly indicates that Strobane-DDT exhibits synergistic action similar to that observed for toxaphene-DDT against resistant boll weevils. In these laboratory tests, Strobane was not so effective either alone or in combination with DDT, as toxaphene against either weevil strain.

Seasonal Tolerance to Toxaphene-DDT.—Dosage-mortality data for boll weevils collected in the field or reared in the laboratory at various times of the year and treated with toxaphene-DDT (2–1) are given in table 3. The LD-50s are presented as milligrams of insecticide per

Table 3.—Effectiveness of toxaphene-DDT (2–1) applied topically to boll weevils collected at various times of the year during 1958 and 1959.

DATE	LD-50 EXPRESSED AS MG. TOXICANT/GM. BODY WEIGHT			
	College Farm	Tabor	Mumford	College Station
Field-collected weevils (120 weevils/test)				
August 11, 1958	0.41	0.70	—	—
August 18, 1958	0.64	—	0.11	—
August 25, 1958	0.36	0.49	0.39	0.53
September 6, 1958	0.29	2.00	—	0.27
October 17, 1958	—	—	0.26	0.27
Square-collected weevils (340 to 480 weevils/test)				
June 1959	—	—	—	0.50
July 1959	—	—	—	0.29
August 1959	—	—	—	0.23
September 1959	—	—	—	0.60
Laboratory-reared weevils (480 to 760 weevils/test)				
December 1958	Resistant strain			0.80
February 1959	Resistant strain			1.05
March 1959	Susceptible strain			0.51

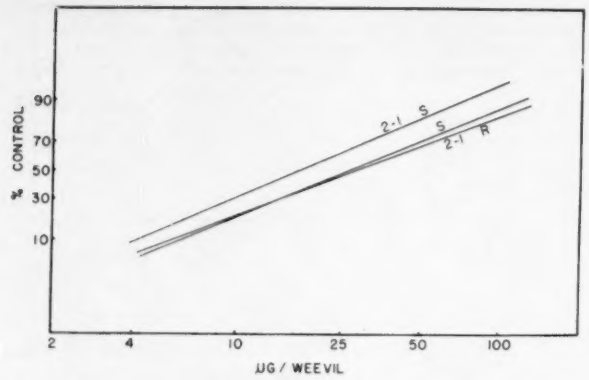

FIG. 5.—Dosage-mortality curves for resistant (R) and susceptible (S) boll weevils treated topically with Strobane alone and Strobane-DDT (2–1) mixture.

gram of weevil weight since many of these weevils were field collected and there was considerable variation in weevil weight. There is little variation in the amount of insecticide required to produce 50% mortality in any of the categories of weevils listed. Rainwater & Gaines (1951) demonstrated that boll weevils were progressively more difficult to control with several chlorinated hydrocarbon insecticides as the cotton growing season advanced. Brazzel & Hightower (1959) reported a seasonal tolerance to toxaphene up to 100-fold in field populations of weevils collected late in the season after the advent of diapause in the population. These results indicate no evidence of seasonal tolerance to toxaphene-DDT (2–1) mixture.

These results show that toxaphene applied in combination with DDT is as effective for resistant weevils as toxaphene alone for susceptible weevils. Since it has been shown that toxaphene apparently does not greatly influence the absorption or metabolism of DDT (Roussel *et al.* 1959), (Lindquist and Brazzel, unpublished data) the increased mortality of resistant weevils with toxaphene-DDT mixtures is apparently not caused by the toxic action of DDT. This supposition indicates that the role of DDT is inactivation of the resistance mechanism in the resistant strain, resulting in toxaphene's being nearly as effective for the resistant strain as for the susceptible strain. This result is in accord with one of the suggested mechanisms by Roussel *et al.* (1959) of interference of toxaphene detoxification by DDT, allowing toxaphene to reach the site of action and possibly the simultaneous interference of detoxification of both chemicals.

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BOOK REVIEW

TYROGLYPHOIDEA (ACARI). FAUNA OF U.S.S.R. ARACHNOIDEA, vol. vi, no. 1, pp. 1–573, by A. A. ZAKHVATKIN. Translated and edited by A. RATCLIFFE and A. M. HUGHES, and published by the American Institute of Biological Sciences, 2000 P. St., N.W., Washington 6, D. C., 1959. Price $10.00 in the U. S. and Canada; $11.00 foreign.

This classic Russian work, which has been unavailable to most workers, is now printed for the first time in an excellent English translation. The mites belonging to the Tyroglyphoidea are serious pests of stored foods—these are the well known cheese mites, grain mites, bulb mites, etc., and this book should be in every entomology library. It is comprehensive, covering all phases of the mites—some of the more important chapters being on Ecology, Economic Importance, Methods of Control, Distribution, and Systematics. The systematic section is divided into three parts—the Tyroglyphidae, the Saproglyphidae, and the Glycyphagidae, the first family containing most of our economic species. Since the original Russian edition was published in 1941, some changes have been made in the taxonomy of these mites. These changes may be found in the works of A. M. Hughes and Phyllis Robertson. According to some acarologists, most of the subfamilies of the Glycyphagidae should be raised to family levels. However, this is of minor importance. The great value of this book is that it has brought together all the information on the Russian and Western European species. The keys are excellent and include males, females, and hypopial forms.

The figures are good although somewhat reduced. The offset printing is of excellent quality.

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Mortality Factors for the Vetch Bruchid, *Bruchus brachialis*[1]

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Abstract

The vetch bruchid, *Bruchus brachialis* Fahr., is an insect pest of European origin that has become one of the major insect pests in the production of seed of hairy vetch. *Vicia villosa* Roth, in the United States.

Mortality data were collected on the development of the insect from egg to emerging adult. Mortality in the egg stage resulted from these factors: eggs lost from the pods (29.7%), death of embryo (8.5%), sterile or nonviable eggs (4.0%), and female bruchids feeding on their eggs (1.7%). In the larval stage, there was a 21.7% mortality of individuals that failed to enter a seed, and 23.5% of the larvae died within the seeds. Very little difference occurred in success of larvae between those that entered the seed through the hilum, or those that entered on the side of the seed. The pupal stage showed no mortality, and less than 1% mortality occurred owing to failure of prepupae to pupate or adults to escape from the seed. The accumulated mortality from egg to adult was 89.32%.

The vetch bruchid, *Bruchus brachialis* Fahraeus, is an insect pest of European origin that has become distributed throughout most of the vetch-seed-producing areas of the United States. In areas where it has become established, this bruchid is usually considered one of the major seed pests of hairy vetch, *Vicia villosa* Roth.

During the course of an investigation of the biological activities of the vetch bruchid, a life table was established for the immature stages. Since only one adult was produced per seed, and a large number of eggs were deposited on the majority of vetch pods, a high rate of mortality during the immature stages was indicated.

Previous observations on mortality of immature stages of the vetch bruchid are reported in literature. Pinckney (1937) obtained a 75% hatch of eggs under field conditions, and a 70% hatch under laboratory conditions. He noted that some larvae died within the pod before finding a seed, or while effecting entrance into a seed. He commented that more than one larval entry occurred per seed, and stated that generally the stronger and more aggressive larvae destroyed the weaker specimens when they met in a seed. Steinhauer (1959) reported an 82% hatch of eggs in laboratory rearing trials, but observed that under field conditions egg mortality ran as high as 50%. Bridwell & Bottimer (1933) found up to seven larval entries per seed, but found that only one adult was produced per seed. Randolph & Gillespie (1958) reported that in only a few instances was there more than one individual per seed.

Experimental Procedure.—The procedure followed in this study was to observe the number of survivors out of a known initial population. It was not possible to measure mortality after adults had emerged from the seeds, but the measurement of all factors was not necessary or practical in order to obtain a useful picture of the delimited population mortality.

Samples for the life-table data were collected from a hairy vetch field near Corvallis, Oregon, during the 1958 season. Immediately following maturity of pods, and prior to dehiscence of the most mature pods, the entire above-ground portions of selected plants were placed in individual boxes for later subsampling. This procedure was necessary to prevent loss of insect eggs from the pods during sampling and subsequent handling.

Each pod sampled was examined and the following data recorded: eggs lost from pod, sterile eggs, eggs with dead embryo, eggs eaten by female bruchid, eggs hatched, and number of larval entrance holes into the pod. The seeds were removed from the pod and the number and position of larval entrance holes (through the hilum or side of the seed) recorded. Each seed was kept separate in a coded paper envelope and retained for adult emergence.

Any attempt to observe larval activities by opening pods and dissecting seeds resulted in death of the individuals exposed. As a result, the discussion of larval mortality factors is based only on collateral observations of material dissected throughout the activity period of the vetch bruchid during 1957 and 1958.

Results and Discussion.—As shown in table 1, 29.7% of the eggs, or 1,286 eggs out of the initial population of 4,333, were lost from the pod. Eggs that dropped from the pod could have exhibited any of the mortality factors for eggs given in table 1. It was impossible to designate these factors after the eggs were lost, but fortunately such designation was not essential to these data. The dropping of eggs from pods, regardless of their condition, was the major mortality factor. Adults brushed off some of the eggs as they crawled about during oviposition, other eggs appeared to be weakly cemented to the pod and fell off shortly after oviposition. Plant movement and plant contact in the field could have brushed off many eggs.

The loss of eggs from the pods was determined by examination of the pods under a binocular microscope for traces of the missing eggs. The presence of a slightly chewed area, somewhat darkened, or remnants of the dried and somewhat shiny adhesive substance indicated a lost egg. If a completed larval entrance hole appeared in the valve, the egg was not scored as lost from the pod because the presence of such an entrance hole indicated that a larva had successfully entered the pod. From 3 to 9 eggs were lost from 135 out of the 188 pods in the sample, and only 3 pods retained all eggs.

Eggs containing a dead embryo accounted for 8.5% of the mortality, or 370 of the initial 4,333 eggs (table 1). The cause of embryo mortality was not determined. Possibly it was a result of some physical factor in the environment or a genetic feature of the particular eggs. This

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Table 1.—Life table for the vetch bruchid based on mortality found in 188 pods with 559 out of 620 seeds infested.

Stage	Number	Mortality Factors	Number Dying	Per Cent Mortality Computed From D/I[a]	Per Cent Mortality Computed From D/S[b]
Eggs	4,333	Eggs lost from pod	1,286	29.7	
		Embryo dead in egg	370	8.5	
		Sterile (non-viable) eggs	173	4.0	
		Egg eaten by female bruchid	73	1.7	
		Mortality in egg stage			43.9
Larvae	2,430	Failure to gain entrance into seed. Instar I	941	21.7	
		Failure to mature in seed. Instar I (late stadia), II, III, IV (early stadia)	1,018	23.5	
		Instar IV (late stadia)	6	0.13	
		Mortality in larval stages			80.8
Pupae	465	None	—	—	
Adults	465	Failure to escape from seed	4	0.09	
		Mortality in adult stage			0.86
Adults emerged	461		—	—	
Accumulated mortality (egg to emerged adult)				89.32	

[a] D/I = number dying divided by number in initial population.
[b] D/S = number dying within a stage divided by total population for that stage.

factor occurred in small numbers among approximately three-fourths of the pods sampled.

Sterile, or nonviable, eggs accounted for a 4% mortality, or 173 eggs out of the initial 4,333 (table 1). The frequency of pods with sterile eggs was low in contrast to the previously mentioned mortality factors. In sterile eggs no embryonic development took place, and the eggs were empty and transparent. More sterile eggs were laid early in the oviposition period than during the peak of oviposition, or later in the season.

The fact that individual females ate their eggs immediately following oviposition accounted for 1.7% of the initial mortality. This phenomenon was first observed during the 1958 season. However, eggs typical of those fed on were noted during the biological studies conducted in Michigan during 1956 and in Oregon during 1957, but it was assumed to be the result of unaccountable predatory insects. There was no explanation for this activity, although some insects are known to require a protein feeding to develop their ovaries and frequently feed on their eggs.

Based on the initial population of eggs, 43.9% mortality occurred in the egg stage, a much greater egg mortality than has been reported in literature. Presumably previous workers calculated per cent of hatch only from those eggs present on the pods, because no mention was made of eggs lost from the pod. Computed in this manner, there was an 80% hatch of eggs remaining on the pods, which fact agrees with the previously cited 75% and 82% egg hatch obtained under field conditions (Pinckney 1937, Steinhauer 1959).

The 2,430 eggs that survived the above mortality factors (table 1) hatched and the larvae entered the interior of the pod through the valves. Only two pods were without larval entrances, and the majority of pods had many more entrances than seeds (average 3.3 seeds per pod).

The initial occurrence of larval mortality within the pod was due to the failure of larvae to gain entrance into a seed. This accounted for a 21.7% mortality of the initial population, or 941 larvae (table 1). This mortality was confined to the first instar larvae. Once inside the pod the larvae crawled about, apparently at random, before gaining entry into a seed. They appeared capable of entering at any point on a seed, including the hilum. Observations indicated that a point of purchase was necessary for a larva to gain entry into a seed. That is, larvae appeared to gain entry where seeds were adjacent to each other, or where seeds were in close proximity to the walls of the valve. Those that entered through the hilum mined first in the funiculus, which afforded them a point of purchase for boring into the seed.

Failure of larvae to mature within the seed accounted for a 23.5% mortality of the initial population. Of the 1,489 larvae that gained entry into the seeds, 1,018 failed to complete their development.

Destruction of one individual by another (as mentioned by Pinckney 1937) was found in only one seed; and then both larvae (third instar) were dead in a common central cavity. However, in some seeds, larvae (second and third instars) were alive in a central cavity and apparently had not attacked one another. Until the tunnels or burrows in the seed opened into a central cavity, there was rarely an instance of tunnels coming into contact with one another. In spite of this lack of physical contact by early instar larvae, many were dead in their isolated burrows. The cause of this mortality was not determined.

There was very little difference in success of larvae between those that entered the seed through the hilum and those that entered at any other point along the side of the seed. A 26% mortality occurred when a single larva entered a seed through the side of the seed (table 2) and was the only individual in the seed; a 28% mortality took place when a single individual entered the seed through the hilum (table 2) and was the only individual in the

Table 2.—The number of vetch bruchids produced from hairy vetch seeds with various combinations of larval entrance positions and numbers.

Number (and Position) of Entrance Holes per Seed		Infested Seeds		
			With Live Adults	
Side of Seed	Through Hilum	Total No.	Number	Per Cent
0	1	57	41	72
0	2	15	14	93
0	3	1	1	100
0	4	1	1	100
1	0	91	68	74
1	1	85	67	78
1	2	19	16	84
1	3	4	3	75
2	0	60	51	85
2	1	65	57	88
2	2	16	12	75
2	3	2	2	100
3	0	40	36	90
3	1	33	27	82
3	2	13	12	92
3	3	5	5	100
4 to 8[a]	0 to 2	52	48	92

[a] Grouped because of small and scattered samples in remaining seeds.

seed. Thus, quite surprisingly, considerable mortality of single individuals was noted within a seed developing free of competition.

The higher the larval population within a seed, the greater the chances for an adult to be produced from that seed (table 2). A similar relationship, of increased population and higher adult yield, existed between the initial number of eggs available for each seed and percentage of seeds producing adults (table 3). These data were obtained by dividing the number of seeds per pod by the number of eggs per pod, and computing per cent of adult yield.

There were 155 seeds that did not produce an adult during the experiment. Of these, 61 were not infested and the remainder showed some feeding activity of larvae. Six contained a dead prepupa, and four contained a dead adult (table 1). Combined, these two mortality factors accounted for less than 1.0% of the total mortality. None contained a dead pupa. Had insect parasites been present,

Table 3.—Per cent of adult yield from seeds with various numbers of eggs on the pods for each seed in the pod.[a]

No. of Eggs for Each Seed in the Pod	No. of Seeds in Sample	Per Cent of Seeds Producing Adults
1– 2	24	29
3– 4	126	51
5– 6	227	78
7– 8	98	76
9–10	59	87
11–12	23	83
13–14	43	88
15–over	20	95

[a] Any fractions of eggs computed to the nearest whole number.

Table 4.—The number of vetch bruchid eggs per pod related to the per cent mortality from egg to adult.

No. of Eggs per Pod[a]	No. of Pods in Sample	Per Cent Mortality from Egg to Adult
1–10	16	84
11–15	31	89
16–20	36	87
21–25	38	88
26–30	33	90
31–35	12	91
36–40	11	90
40–over	11	92

[a] The number of eggs per pod arranged by classes to increase sample size of pods for computing weighted mortality percentages.

parasitism would have comprised an additional mortality factor.

The total mortality of 89.32% (table 1) was only slightly greater than a calculated mortality would have been for the conditions of the experiment. Based on 4,333 eggs in the initial count, and a total of 620 seeds in the sample, a calculated mortality would have been 85.7%. However, a calculated mortality assumes that each seed was infested and that each seed produced an adult. Under the conditions of the experiment, one adult was produced for each 10 eggs deposited on the pods of hairy vetch.

Although there was an increased adult yield from an increased number of eggs per seed (table 3), the total insect mortality per pod remained independent of the egg population per pod (table 4) and approximated the 90% mortality that was obtained for the experiment. The mortality percentages in table 4 are calculated from the

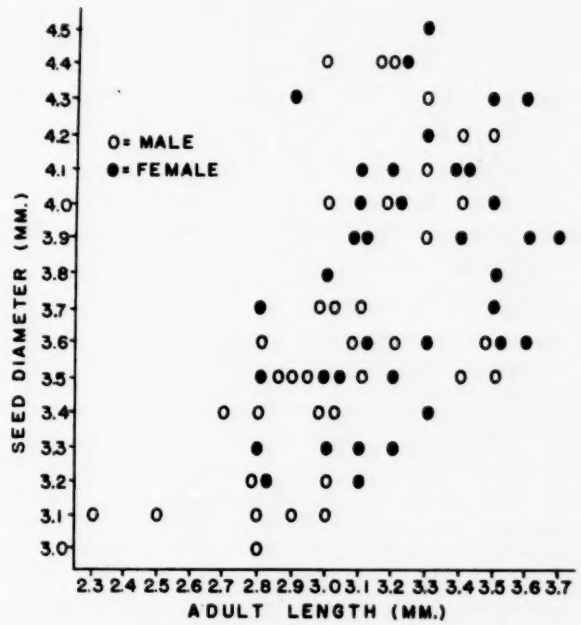

FIG. 1.—Scatter diagram showing adult size (by sexes), of the vetch bruchid in relation to diameter of hairy vetch seeds from which adults emerged.

total eggs per pod, arranged by classes, and adults produced from these pods. Of biological significance is the fact that this mortality existed in individual pods. The number of eggs per pod ranged from 4 to 66.

The size of the mature vetch seed appeared to have no relationship to the success or failure of larvae to complete development. Bridwell & Bottimer (1933) noted that small, poorly developed seeds produced small adults. Similar observations were made during this study (fig. 1), although there was considerable variation in size among bruchids produced from well developed seeds.

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Yield and Quality of Mushrooms from Beds Treated with Lindane[1]

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ABSTRACT

Studies were made of the tolerance of mushrooms to lindane applied in a water suspension to the bearing beds for the control of mushroom flies, chiefly the sciarid fly, *Bradysia fenestralia* (Zett.), and the phorid fly, *Megaselia agarici* Lintner. Dosages of 0.5 pound per acre or less did not affect the yield, but 1 pound or more tended to depress yield. Neither dosage left appreciable residues on mushrooms harvested 5 days after application. Only slight occasional off-flavors were found in canned mushroom pieces and cream of mushroom soups made from mushrooms from beds treated with 0.25, 0.5, and 1 pound of lindane per acre 7 days or more before harvest. Samples from mushrooms harvested 3 days after treatment had musty off-flavors, which made both the pieces and the soup inferior in eating quality.

One of the first uses of lindane after its introduction in 1949 was in the control of mushroom flies, chiefly the sciarid fly, *Bradysia fenestralia* (Zett.), and the phorid fly, *Megaselia agarici* Lintner. Technical BHC had previously been reported to be effective against these flies (Thomas 1947, Shanahan 1948). Lindane was included with other insecticides in routine studies by the Entomology Research Division at Beltsville, Maryland, on the tolerance of mushrooms to insecticide drenches. In the fall of 1952 reports were received of undesirable flavors in canned mushrooms thought to be caused by the use of lindane on the growing crop. Increased emphasis was therefore given to lindane and the studies were expanded to include palatability tests. Studies conducted during the 1951–52 and 1952–53 crop seasons to determine the yield and residues of mushrooms treated with lindane, and the flavor and general acceptibility of the marketed product are reported herein.

METHODS.—In these studies no appreciable infestation of mites or insects was encountered, apparently owing to isolation of the experimental house. Otherwise the mushrooms were grown under simulated commercial conditions in tiers of beds 5 feet wide and five beds high on each side of the house. A 25% lindane wettable powder was applied in a water suspension by means of a sprinkling can with a fine cone nozzle and a spout long enough to reach under and across the beds. This equipment made it possible to drench uniform 16-square-foot plots arranged in four randomized blocks, one block on each of the top four tiers. For each treatment 1 liter of suspension was applied on each plot and an equal quantity of water to the check plots.

The mushrooms were harvested two or three times each week, examined for visible injuries, and weighed. Samples were also cooked in various ways in the homes of members of the entomology staff and examined for flavor and general acceptibility. Other samples were analyzed for lindane residues by the colorimetric method of Schechter & Hornstein (1952). In 1953 5-pound samples were canned and subjected to flavor evaluations by a specially trained panel in the Human Nutrition Research Division.

RESULTS.—*Experiments in 1951–52.*—During the 1951–52 season exploratory tests were made of 0.9- and 3.6-pound dosages of lindane per acre. The first application was December 21, or 5 days before the first picking of mushrooms. Additional applications were made January 5, between the first and second flushes, and February 13, between the second and third flushes. A total of 36 pickings was made between December 26 and April 16. The yield of mushrooms was 1.35 pounds per square foot from the plots treated with 3.6 pounds of lindane per acre, 1.72 pounds from those receiving the 0.9-pound dosage, and 2.27 pounds on the check plots. Some of the mushrooms from plots receiving the higher dosage were deformed to give a rose-comb effect. No undesirable flavor was noted by the entomology staff in the informal tests in their homes.

As shown in table 1, the residues were well below the established tolerance of 10 p.p.m. of lindane on mushrooms harvested 5 or more days after application.

Experiments in 1952–53.—Several dosages of lindane were applied as in the earlier tests except that each plot measured 18 square feet. The first application was made December 3, 2 days before the casing soil was applied; the second on December 30, when the first flush of mushrooms was in the pinhead stage of growth; and the third 17 days later, between the first and second flushes. As

[1] Accepted for publication January 13, 1960.

[2] Retired June 1953.

[3] F. F. Smith, A. L. Boswell, L. B. Reed, and R. H. Carter (retired), Entomology Research Division, ARS, made valuable contributions to this work.

Table 1.—Lindane residues on washed mushrooms grown in plots sprinkled with lindane suspension.

LINDANE PER ACRE (LB.)	DATE OF LAST APPLICATION	DAYS FROM LAST APPLICATION TO HARVEST	P.P.M. OF RESIDUE (CORRECTED FOR CHECK)
	Experiments in 1951–52		
3.6	Dec. 21	5	0.5[a]
		12	.5
			.2[a]
	Jan. 5	23	.4[a]
	Feb. 13	16	.7[a]
		30	.6
0.9	Dec. 21	5	0[a]
		12	0
			0[a]
	Jan. 5	23	.1[a]
	Feb. 13	16	.1
		30	0
	Experiments in 1952–53		
1.0	Dec. 30	9	0.3
	Jan. 16	5	.2
		20	.1
0.5	Dec. 30	9	Lost
	Jan. 16	5	.1
		20	.1
.25	Dec. 30	9	.1
	Jan. 16	5	.1
		20	< .05

[a] Not washed.

shown in table 1, very little residue was found on the harvested mushrooms. The yields were as follows:

Pounds of lindane per acre	*Pounds of mushroom per square foot*
1	1.71
0.5	1.90
0.25	1.86
None	1.69
L.S.D.	.07

A 5-pound sample of mushrooms was taken from each of the treated plots during each of the first four flushes of harvest—January 6 and 19 and February 3 and 26—and shipped to a commercial canning firm in Camden, New Jersey, for processing. Each sample was a composite from the four replicates. Half of each sample was canned as mushroom pieces packed in water, and the other half as cream of mushroom soup. The canned foods were rated for flavor and general acceptability by the Human Nutrition Research Division.

The mushrooms and soup, all in No. 1 tin cans, were held in storage at room temperature for approximately 6 months. The cans were then opened and the contents heated in identical covered pyrex glass saucepans. The mushroom pieces were heated to boiling in their own juice; each can of soup was thinned with 300 grams of distilled water and then heated to boiling.

A 4×4 lattice design (Cochran & Cox 1950) was used for the mushroom pieces in this study and a separately randomized lattice for the soup. In each 2 subsamples from each of the 16 samples were rated in 8 judging sessions, 4 subsamples being served at each. The cooking time was arranged so that individual samples were ready for serving at 3-minute intervals. They were served warm in coded glass dishes to a panel of five persons, all with some previous experience in rating foods exposed to insecticide treatments during production. The samples were scored for absence or presence of off-flavor on a 5-point scale, 5 indicating no off-flavor and 1 very strong off-flavor; any detected off-flavor was named. They were also rated for general acceptability, an overall rating based on color and texture as well as flavor, on a 5-point scale with 5 denoting very good and 1 very poor products. Before undertaking tests of the experimental samples, panel members were given experience in recognizing the natural flavor of mushrooms by tasting untreated samples of mushroom pieces and mushroom soup.

As shown in table 2, only slight off-flavors were found in any samples prepared from mushrooms of the first, third, or fourth harvests. From these harvests there were no significant differences among scores given the insecticide-treated samples or between them and the untreated check, and notations of mustiness were too few to be considered indicative of serious off-flavor.

Flavor scores for the pieces of mushrooms from all

Table 2.—Palatability scores for canned mushroom pieces and cream of mushroom soup from mushrooms grown in beds treated with lindane or in untreated beds.

HARVEST	DAYS FROM LAST APPLICATION TO HARVEST	FLAVOR				GENERAL ACCEPTABILITY			
		Lindane Dosage per Acre			Untreated Check	Lindane Dosage per Acre			Untreated Check
		1 Lb.	0.5 Lb.	0.25 Lb.		1 Lb.	0.5 Lb.	0.25 Lb.	
		Mushroom pieces							
First	7	4.4	4.0	4.4	3.9	3.3	2.9	3.2	3.1
Second	3	3.4	3.2	3.2	3.8[a]	2.5	2.4	2.6	3.0
Third	18	3.8	4.3	4.3	3.9	2.8	3.4	3.1	2.9
Fourth	41	3.9	4.4	4.0	4.0	3.0	3.1	3.2	3.0
		Cream of mushroom soup							
First	7	4.3	4.4	4.4	4.2	3.9	4.1	4.2	3.9
Second	3	3.4[a]	4.6	4.7	4.9	3.1[a]	4.2	4.2	4.4
Third	18	4.8	4.6	4.2	4.4	4.3	4.1	4.0	3.9
Fourth	41	4.3	3.8	4.3	4.6	3.9	3.5	4.0	4.1

[a] Significantly different at 5% level from scores of other samples from the same harvest.

treated samples of the second harvest were significantly lower than the score for the check sample, but were not different from each other. In about half the judgments mustiness was noted in samples from the three treated lots in the second harvest. The scores for the mushroom pieces from this harvest were also lower than those for samples having the same treatment in other harvests. In soup, the scores for both flavor and general acceptability of mushrooms from the second harvest grown with lindane at 1 pound per acre were significantly lower than the scores for the other treated samples and for the check. Since the second harvest was made the shortest time (3 days) after the last insecticide treatment, any effect on flavor might be expected to be most apparent in these samples.

A panel of eight people from the company that canned the mushrooms also tasted these products. They too found that all samples from the second harvest were off-flavored. In addition, they reported slight off-flavor in mushrooms from the first and fourth harvests and objectionable off-flavors in mushrooms from the third harvest from plots treated at the 1-pound rate.

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Chloroplast Number in Leaves of Normal Wheat Plants and Those Infested with Hessian Fly or Treated with Maleic Hydrazide[1,2]

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ABSTRACT

Chloroplasts were counted in leaves of normal wheat plants, those infested with hessian fly (*Phytophaga destructor* (Say)), and those treated with maleic hydrazide. The chloroplast number decreased in the outer leaves and increased in the inner leaves of the treated plants. In the hessian fly infested wheat plants, the chloroplast counts increased in both inner and outer leaves over those of the normal plants. It appeared that the dark-green color of the inner leaves of hessian fly infested wheat was due to increased chloroplast numbers. It appeared further that the dark-green color of the outer leaves of maleic hydrazide treated plants was due to a greater concentration of chlorophyll per chloroplast.

Miller *et al.* (1958) reported that the dark blue-green color of the center leaves of plants infested with the hessian fly (*Phytophaga destructor* (Say)) may be due to the substantially higher concentrations of chlorophyll a and b in these leaves compared with concentrations in outer leaves of the same plants or with those of comparable leaves of uninfested plants. The center leaves of uninfested plants contained less chlorophyll a and b than the outer leaves of the same plants. The ratios of chlorophyll a to b in both inner and outer leaves were higher for uninfested than for infested plants.

The application of maleic hydrazide to certain plants produces effects similar in some respects to those resulting from hessian fly infestation of wheat. Moore (1950) sprayed the leaves of various plants with maleic hydrazide and observed in some a transient intensification of the green color in leaves of stunted plants, a localized accumulation of anthocyanins or other nongreen pigments, and an interference with water absorption apparently caused by the death of root tips. Samborski & Shaw (1957) observed that maleic hydrazide inhibited the growth of Khapli emmer. No data on the actual pigment concentrations were presented and it was not clear whether the intensification of the green color was owing to accumulations of chlorophyll a and b or to a higher density of chloroplasts per unit area.

The objective of the present study was to determine the relationship between the number of chloroplasts and the dark-green color that develops when plants are infested with hessian fly or treated with maleic hydrazide.

MATERIALS AND METHODS.—Comparable lots of infested and uninfested Tenmarq and Pawnee wheat plants were grown in hydroponic solution (Grossman *et al.* 1957). Pawnee and Tenmarq wheat plants also were grown in soil in 15″×22″ flats and were watered with 1,750 ml. of 0.1% maleic hydrazide at the time of plant emergence and again after 3 days. A second planting of Pawnee and Tenmarq wheat in flats was watered with 1,750 ml. of 0.1% maleic hydrazide when the secondary leaves became longer than the primary leaves, 13 days after planting, and again 3 days later.

Center and outer leaves of the plants were harvested when the plants were about 30 days old. Five-gram samples of the fresh leaves were placed in plastic bags

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[2] The authors gratefully acknowledge suggestions of Mr. C. O. Johnston, Kansas State University, concerning treatment of wheat plants with maleic hydrazide.

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and frozen immediately at −20° C. Moisture was determined by drying additional samples at 130° C. for 5 hours.

Analysis for Lipid-soluble Plant Pigment.—Chlorophyll was determined by the official method of the Association of Official Agricultural Chemists (1950), using the 5-gram frozen samples. Carotene and xanthophyll were determined by the method of Gugliemelli & Mitchell (1958), as adapted by Miller *et al.* (1958).

Preparation of Chloroplast Suspensions.—Chloroplast suspensions were prepared according to the method of Arnon *et al.* (1956). Five-gram samples of fresh wheat leaves were cut into ½-inch pieces and placed in a 50-ml. Sorvall Omnimixer cup containing 15 ml. of chilled 0.35 M. sodium chloride. The cup was surrounded by an ice bath and the mixer was allowed to operate for 5 minutes, after which the supernatant liquid was squeezed through a double layer of cheesecloth. Following a washing with 5 ml. of fresh, cold 0.35 M. sodium chloride solution, the pulp was discarded. The combined filtrate was centrifuged for 10 minutes at 0° C. at 200×g. to remove leaf debris and whole cells. The green supernatant liquid was carefully decanted and centrifuged for 20 to 30 minutes at 0° C. at 1,000×g. to sediment the whole chloroplasts. The yellow supernatant liquid containing broken chloroplast fragments and smaller cellular particles was decanted and discarded. The whole chloroplasts were resuspended by gentle agitation after the volume was increased to 3 ml. with ice-cold 0.35 M. sodium chloride solution. The suspension was drawn up to the 0.5 mark of an edax white blood cell diluting pipette and immediately diluted to the 11 mark with a 1% solution of malachite green. This technique resulted in a 1 to 20 dilution of chloroplasts which took up the green stain reasonably well within 3 minutes after mixing. No attempt was made to determine the proportions of chloroplasts recoverable in the whole chloroplast fraction. The count conceivably could be influenced by changes in fragility owing to the effect of hessian fly infestation or to maleic hydrazide treatment.

Counting the Chloroplasts.—The counting of chloroplasts was based on the procedure for blood counts. The prepared chloroplast suspension was placed on a Spencer Bright Line Haemacytometer with improved Neubauer ruling. Chloroplast counts were made by use of the same procedures for dilutions, counting, and calculations employed in standard total white blood cell determinations (Methods for Laboratory Technicians 1941, Seiverd 1958).

The initial chloroplast counts were made by preparing microscopic slides according to the histological technique for the examination of plant structures (Johansen 1940, Thomas 1958). The chloroplasts were attached to the slides with Haupt's adhesive (Johansen 1940). Several stains and staining techniques were applied. Such techniques as Eosin-hematoxylin, simple Gram, fast green-safranin "O," acid-fuchsin-aurantia, and malachite green were investigated. Malachite green was found to be most useful.

RESULTS AND DISCUSSION.—*Effect of Maleic Hydrazide on Pigment Concentration in Wheat Leaves.*—A definite inhibition of growth owing to maleic hydrazide treatment was apparent after 10 days. At this time treated plants were darker green than the untreated ones. Unlike those of hessian fly infested plants, however, the center leaves were narrow and lighter green than the outer leaves.

The chlorophyll, carotene, and xanthophyll analyses of the inner and the outer leaf tissue of treated and untreated plants are summarized in table 1. The pigment concentrations in both inner and outer leaves of treated plants were consistently higher (on a fresh-weight basis) than those in comparable leaves of normal plants. Unlike hessian fly infested plants (Miller *et al.* 1958), the outer leaves of treated plants had essentially the same amount of pigment as the inner leaves of the same plants.

Chloroplast Counts.—Initial chloroplast counts made by the flat-stain technique were difficult because of clumping, but information on size and shape was obtained. The chloroplasts were essentially round and ranged from 5 to 9 μ in size. The size range suggested that the well-known haemocytometer method for counting blood cells might be applied. The chloroplast counts obtained by the latter method are shown in table 2. In plants treated with maleic hydrazide, the number of chloroplasts in the outer leaves decreased, and the number of chloroplasts in the inner leaves increased. In leaves infested with hessian fly, with one exception, chloroplasts increased in both inner and outer leaves. Although treatment with maleic hydrazide or infestation with hessian fly might have changed the fragility of the chloroplasts, the comparisons of inner and outer leaves of the same plant would not be influenced by this possibility. The low count for the hessian-fly-resistant variety M.O.O.T. (table 2) may be due to a peculiar characteristic of its chloroplasts. The M.O.O.T. chloroplasts were larger and the leaves were lighter green than those of other varieties studied. The inner leaves of the infested M.O.O.T. variety did not attain the deep, dark-green color of the other varieties. Instead, those of the infested plants tended to become lighter green with time.

The difference in the number of chloroplasts in the treated and the infested plants might be attributed to different actions of the two agents on the growth of the

Table 1.—Effect of maleic hydrazide on pigment concentration of wheat leaves.

TISSUE FROM LEAVES	DRY WEIGHT (%)	MG./100 GM. FRESH WEIGHT			
		Chlorophyll		Carotene	Xanthophyll
		a	b		
Tenmarq control:					
Inner	20.7	118	47	11.0	27.9
Outer	17.8	89	36	8.0	19.8
Treated Tenmarq:					
Inner	25.6	140	59	13.3	32.1
Outer	20.6	140	60	13.5	29.3
Pawnee control:					
Inner	19.3	123	49	11.8	26.3
Outer	16.5	120	49	12.0	25.9
Treated Pawnee:					
Inner	23.7	150	63	14.2	33.0
Outer	20.7	146	61	14.9	31.8

Table 2.—Effect of maleic hydrazide treatment and hessian fly infestation on chloroplast counts of wheat leaves.

VARIETY	TREATMENT	TISSUE FROM LEAVES	CHLOROPLAST COUNT (1,000/ GM. FRESH WEIGHT)
Pawnee[a]	Maleic hydrazide	Treated, outer	1,260
		Untreated, outer	2,025
	Maleic hydrazide	Treated, inner	1,245
		Untreated, inner	210
Tenmarq[a]	Maleic hydrazide	Treated, outer	2,550
		Untreated, outer	2,865
	Maleic hydrazide	Treated, inner	1,605
		Untreated, inner	915
M.O.O.T.[b,c]	Hessian fly	Infested, outer	120
		Uninfested, outer	75
	Hessian fly	Infested, inner	30
		Uninfested, inner	60
Rival[b]	Hessian fly	Infested, outer	170
		Uninfested, outer	100
	Hessian fly	Infested, inner	340
		Uninfested, inner	190
Tenmarq[b]	Hessian fly	Infested, outer	800
		Uninfested, outer	140
	Hessian fly	Infested, inner	1,000
		Uninfested, inner	100

[a] Grown in soil in flats.
[b] Grown in hydroponic culture medium.
[c] M.O.O.T. = Marquillo-Oro × Oro-Tenmarq (C.I. 12406).

wheat plant. Maleic hydrazide is a growth inhibitor which disrupts cell metabolism with a thickening of the cell walls at distal ends and a clearing of the green matter in the center portion of the cell. Growth inhibition seems to move from the outer layer inward and is followed by general breakdown and drying up of plant tissue. The roots of the plant cease to grow when the plant is treated with maleic hydrazide.

When wheat plants were infested with hessian flies the chloroplast count tended to increase. The inner leaf of the wheat plant became dark green and ceased to grow but the plant tissue did not dry up. The infestation tended to move from inside the plant outward. The roots of the hessian-fly-infested wheat plant did not cease to grow immediately as did those of the maleic-hydrazide-treated wheat plants.

The inner and outer leaves of the maleic-hydrazide-treated wheat plants were dark blue-green. The deepest coloration appeared in the outer leaves although the chloroplast count decreased. This deep color appeared to be due to a greater amount of chlorophyll per chloroplast. The dark-green coloration of the inner leaves of the hessian-fly-infested wheat plants appeared to be due to increase in the number of chloroplasts.

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A Fumigant Vaporizer Unit for Use in Laboratory Experiments[1]

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ABSTRACT

The vaporizer unit described and illustrated in this paper proved to be satisfactory for use in applying microquantities of vaporized fumigant mixtures in known proportions, a difficult problem in laboratory testing. A wide range of fumigants can be vaporized with this unit, but the principal use made by the authors was to vaporize fumigants with high boiling points so that the fumigant was introduced into the fumigation chamber in the gaseous form.

Many of the candidate compounds evaluated for their usefulness as grain fumigants vary widely in their physical and chemical properties and require different methods of application.

In tests conducted by the authors, a liquid fumigant was introduced into the test chamber in one of two ways: applied to an absorbent material and allowed to vaporize by its own vapor pressure, or in a gaseous form.

The application of microquantities of vaporized fumigant mixtures in known proportions is a difficult problem

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[3] On military leave.

[4] This laboratory is a field station of the Stored-Product Insects Section, Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

[5] Leon H. Hendricks assisted in the work reported here.

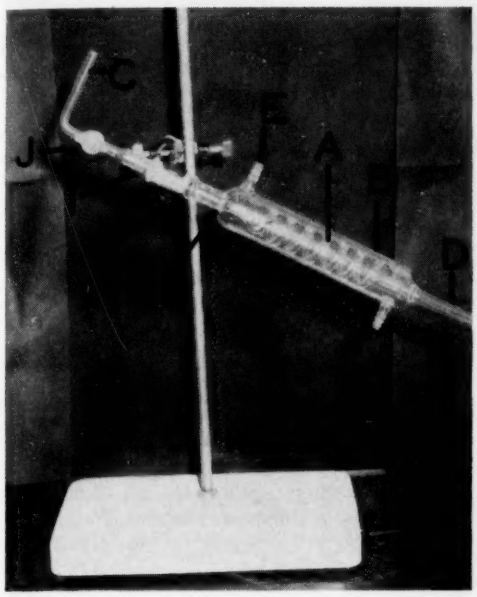

FIG. 1.—Vaporizer with stopcock in place. (See text for explanation of lettered parts.)

in laboratory testing. One of the more satisfactory ways of handling the fumigant is to measure the dosage as a cold liquid, confine it in a nonabsorptive container, and vaporize it into the fumigation chamber by the use of heat. However, care must be taken not to affect the temperature in the fumigation chamber. The vaporizer unit described and illustrated in this paper proved to be satisfactory for this purpose.

The unit (hereafter called a vaporizer) consisted of a Graham condenser of pyrex glass having a jacket approximately 200 mm. long and 40 mm. in diameter (fig. 1, *B*), and open at both ends (fig. 1, *E* and *F*), for the circulation of steam; a coiled conductor tube 8 mm. in diameter sealed within the condenser (fig. 1, *A*), with an outer T-joint (No. 19/38) (fig. 1, *G*) at the upper end (fig. 1, *H*); and a 90° connecting tube (fig. 1, *C*) with a stopcock (fig. 1, *J*) attached to an inner No. 19/38 T-joint (fig. 1, *I*), for the introduction of the liquid fumigant. Short lengths of polyethylene and Tygon plastic tubing were used to connect the vaporizer vent (fig. 1, *D*) to the fumigant chamber.

The unit was mounted on a burette stand at an angle of about 60° (fig. 1) to prevent the liquid fumigant from dropping directly through the coil into the fumigation chamber before being vaporized. Steam was generated by boiling distilled water in a 500-ml. glass distillation flask (fig. 2, 1*A*) on a Bunsen burner. The vent of the flask was attached to the upper opening in the steam jacket of the vaporizer by a Tygon tube (fig. 2, 1*B*). The steam which bathed the condenser coil caused the liquid fumigant to vaporize quickly and its own pressure forced it into the fumigation chamber. By observing the progress of the liquid fumigant through the glass the operator could determine when the fumigant dosage was completely vaporized. Immediately after each use the vaporizer was tested with a halide-leak detector (fig. 2, 1*C*) to determine if all of the gaseous fumigant had been forced from it into the fumigation chamber.

A wide range of fumigants can be vaporized with this unit, but the principal use made by the authors was to vaporize fumigants with high boiling points such as ethylene dibromide (boiling point 131.6° C.), so that the fumigant was introduced into the fumigation chamber in the gaseous form.

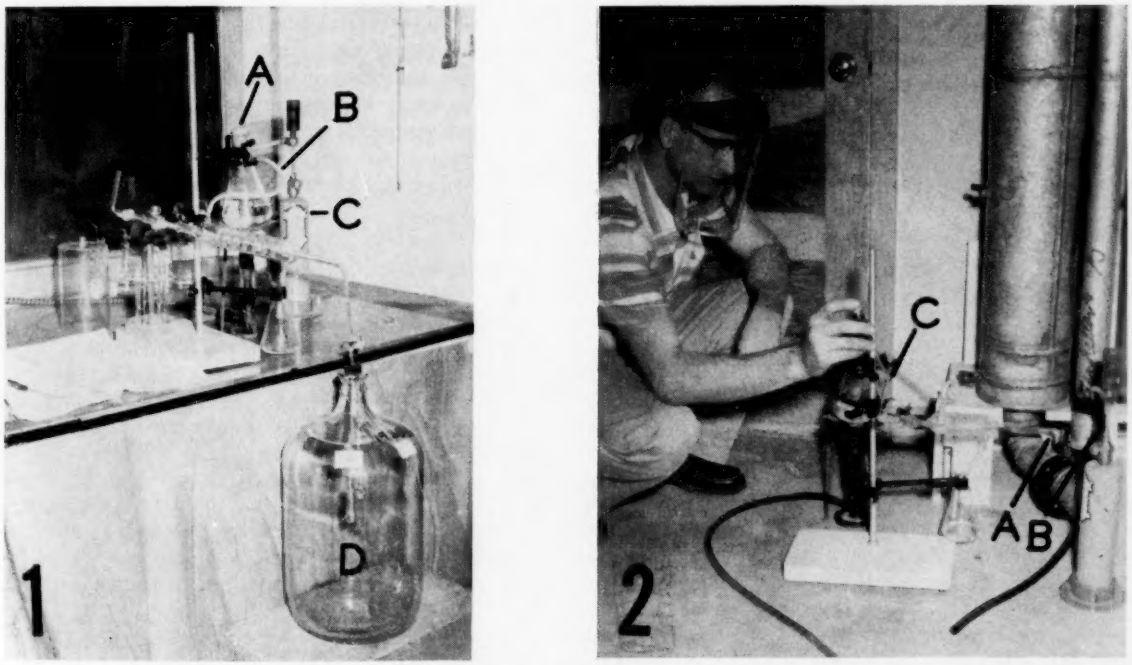

FIG. 2.—*1*, Vaporizer connected to bottle; *2*, vaporizer connected to recirculator. (See text for explanation of lettered parts.)

The vaporizer was used under two sets of conditions. First, it was attached to a partially evacuated (15 inches of mercury) 5-gallon glass bottle (fig. 2, 1*D*) and the vaporized fumigant was pulled through the vaporizer by releasing the vacuum in the bottle. The unit was removed as soon as the vacuum was satisfied. Second, the vaporizer was used to apply the fumigant in laboratory recirculators filled with wheat. The exhaust side of the unit was attached to an opening in the return duct of the recirculator on the intake side of the fan (fig. 2, 2*A*). Negative pressure, causing a slight vacuum in the vaporizer coil, aided in pulling the fumigant vapors into the fumigation chamber. Immediately after the liquid fumigant was introduced, a short polyethylene plastic tube was attached to an opening in the exhaust side of the recirculator fan (fig. 2, 2*B*) and attached to the glass tube (fig. 2, 2*C*) above the stopcock. This arrangement set up a recirculation through the vaporizer unit and aided in washing the gas into the fumigation system. The vaporizer was left attached to the recirculator for 5 minutes to give ample time to vaporize all of the liquid.

Under the conditions tested, the average temperature rise in the empty 5-gallon bottles was about 3° F. No rise in temperature was detected in the wheat-filled recirculators. Two formulations of ethylene dibromide and methyl bromide were tested with no detectable loss of methyl bromide and with complete volatilization of the fumigant in about 30 seconds.

Some precautions to be taken in the use of the vaporizer are:

1. Cool the stopcock and glass tube at the top of the vaporizer before the fumigant is applied in order to prevent premature volatilization.
2. Set the condenser at an angle to prevent the liquid from passing through without being completely vaporized.
3. Keep flammable fumigants away from the open flame of the Bunsen burner, and the electric motors of the fumigation apparatus.
4. Use some method, such as the use of the evacuated bottle, to clean the vaporizer coil of all of the fumigant vapor.

The Effect on Foraging Behavior of Moving Honey Bee Colonies to Crops Before or After Flowering Has Begun[1]

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ABSTRACT

Some colonies of honey bees (*Apis mellifera* L.) were taken to crops before flowering, and others after flowering had begun. Counting bees entering their hives established the relative foraging populations of the two groups; marking bees leaving the hives of the two groups with different fluorescent powders enabled the proportions of them captured on the crops to be ascertained; hence the relative proportions of foragers of the two groups visiting the crops could be calculated. All six experiments showed that the proportion of foragers visiting the crop was increased by delaying the moving of their colonies into it until after flowering had begun.

Free (1959) found that the proportion of honey bees (*Apis mellifera* L.) visiting crops of field beans (*Vicia faba*) and opium poppies (*Papaver somniferum*) was higher when their colonies were moved to crops that had begun to flower than when moved before any flowers had opened. Confirmation of these results for other crops is reported in this paper.

METHODS.—In each experiment one group of colonies (A) was taken to a crop before it flowered and a second group (B) after flowering had begun. In experiments on peach, sweet cherry, and apple, the species concerned was not flowering in the area where Group B colonies were previously present. In the other experiments (birdsfoot trefoil, lucerne and red clover) the species concerned may have been flowering within foraging range of Group B colonies' former location but not in abundance nearby.

Bees were marked using the technique developed by Musgrave (1949) and Smith & Townsend (1951). About 30 minutes after the second group of colonies was placed at the crop, marking blocks covered with fluorescent powder were fitted to the hive entrances of both groups so that bees leaving or entering became dusted with the powder. Fluorescein powder was used for one group and rhodamine for the other; these compounds appear greenish-yellow and yellowish-orange, respectively, in ultraviolet light. Bees foraging on the crop were captured individually in vials, and later moistened with water and examined under ultraviolet light to determine from which group of colonies they had come.

To find the relative foraging populations of the two groups colonies were paired off according to population when possible and simultaneous counts made of the bees returning to each colony of a pair during 5-minute periods; a minimum of three counts per colony per day was made.

RESULTS.—The relative proportions of the bees of each group that foraged on the crops concerned were calculated from the figures obtained (table 1). In each experiment the relative proportion of bees of Group B was greater than that of Group A.

Because, in general, Group A bees were foraging farther afield than Group B and probably made foraging trips of greater duration they would have been counted less frequently at their hive entrances. Hence the relative proportions of bees of Group B/Group A foraging on the crop was, if anything, greater than the data imply.

Peach Experiment (Sandhills' Research Station, North Carolina).—Group A (eight colonies) was moved to 100 acres of peach trees on February 26 and Group B (eight

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[2] Work done while on secondment from Bee Department, Rothamsted Experimental Station.

Table 1.—Numbers of honey bees entering hives and captured on crops.

Crop	Date	Sum of the Mean No. of Bees Entering Each Hive per 5-Minute Period		Proportion (x) of Bees Group B/ Group A Entering Hives	No. Bees Captured on Crop		Proportion (y) of Bees Group B/ Group A Captured on Crop	Relative Proportion (y/x) of Bees Group B/ Group A Foraging on Crop
		Group A[a]	Group B[a]		Group A	Group B		
Peach (*Prunus persica*)	March 17	1,144	736	0.64	38	56	1.47	2.30
	March 19	1,199	881	0.73	45	89	1.98	2.71
Sweet Cherry (*Prunus avium*)	May 9	755	859	1.14	30	79	2.63	2.31
	May 10	906	939	1.04	35	49	1.40	1.35
	May 11	992	1,129	1.14	52	67	1.29	1.13
Apple (*Pyrus malus*)	May 21	1,530	1,611	1.05	21	149	7.10	6.76
	May 22	—	—	—	12	40	3.33	3.17
	May 24	—	—	—	55	92	1.67	1.59
Bird's-foot trefoil (*Lotus corniculatus*)	June 8	775	238	0.31	27	107	3.96	12.77
	June 10	789	809	1.03	58	87	1.50	1.46
Lucerne (*Medicago sativa*)	Aug. 11	773	590	0.76	21	101	4.81	6.33
	Aug. 12	856	1,248	1.46	15	65	4.33	2.97
	Aug. 13	1,151	1,545	1.34	16	49	3.06	2.28
	Aug. 14	543	1,178	2.17	10	28	2.80	1.29
Red clover (*Trifolium pratense*) & Lucerne	Aug. 18	746	981	1.31	11	35	3.18	2.43
	Aug. 20	1,097	1,417	1.29	33	44	1.33	1.03

[a] Colonies of Group A present at crop before flowering and colonies of Group B taken to crop after flowering had commenced.

colonies) moved after dark on March 16 when about 5% of the peach flowers were open. Bees were collected from the flowers on March 17 and 19 on a predetermined course over about 7 acres of orchard near the colonies. The intention was to take not more than two bees from each tree at each inspection; this was done in the apple and sweet cherry experiments but to capture sufficient bees during the peach experiment it was necessary to capture as many as possible from those trees on which they were relatively numerous. The proportion of Group A/Group B bees captured did not vary at different distances from the hives within the 7 acres concerned.

Conditions were unsuitable for bee flight on March 18. The ratio of the relative proportions of bees of Group B/Group A foraging on the crop did not decrease from March 17 to 19.

Sweet Cherry Experiment (Vineland Experimental Station, Ontario).—Group A was moved into a 6-acre sweet cherry orchard on April 27 and Group B early on May 9 when about 25% of the flowers were open. There were six colonies in each group. In this and subsequent experiments the entire area of the crop concerned was sampled. By the second and third days of the experiment, when the trees were in full flower, the relative proportions of bees of the two groups on the crop had become similar.

Apple Experiment (Vineland Experimental Station, Ontario).—Group A (six colonies) was placed in an 8-acre orchard on May 10, and Group B (six colonies) on May 21, when approximately 20% of the flowers were open. In this orchard, as well as in the peach and sweet cherry orchards referred to, trees of several different varieties were present. Bees entering the hives were counted on the first day only and the counts used to calculate the relative proportions of bees of the two groups foraging on the crop during subsequent days. Even on the last day, when most of the apple flowers had withered, proportionally more bees of Group B than of Group A visited the crop. No bees flew on May 23.

Bird's-foot Trefoil Experiment (Brampton, Ontario).—Group A (four colonies) was moved to a 3½-acre field of bird's-foot trefoil on May 26 and Group B (four colonies) on June 8, when the crop was flowering profusely. Relatively few bees foraged from Group B on their day of arrival, probably because of overheating of the colonies on the journey. Unfavorable weather prevented continuation of the experiment after June 10.

Lucerne Experiment (Guelph, Ontario).—A 10-acre lucerne field, from which one cutting had been taken, was used. Group A (four colonies) was placed in it on July 27 before any flowers of the second growth had appeared, and Group B (four colonies) put beside them on August 11, when about 10% of the flowers were open.

The ratio of the relative proportion of Group B/Group A foraging on the crop decreased until by the fourth day it approached unity. The number of bees captured per hour also decreased; most of the bees were probably visiting a red clover field one-half mile away. Lack of storage space in the combs of Group A colonies probably accounts for the relative reduction in the number of their foragers on the last day of the experiment.

Red Clover and Lucerne Experiment (Guelph, Ontario). Group A (four colonies) was placed in a 12-acre field of second growth red clover with some lucerne on July 27, when no flowers were open, and Group B (four colonies) on August 17, when flowering was at about its peak. Observations were made on August 18 and 20 only; by the latter date approximately equal proportions of bees of the two groups were foraging on the crop.

DISCUSSION AND CONCLUSIONS.—Because, at the beginning of each experiment, a higher proportion of bees of Group B visited the experimental crop, it is concluded that bees of Group A had found other flowers before those of the experimental crop opened and continued to visit them when the latter came into flower, whereas bees of Group B found the experimental crop first because it was flowering close to their hives when they arrived. The much greater proportion of bees of Group B than A foraging on the experimental crop in some experiments than in others was probably due to variations in the relative attractiveness and distance of competing crops.

The ratio of bees of Group B/Group A captured on the crop usually decreased during an experiment. The present

method could not show whether this fact was because increasing numbers of bees of Group B foraged on more attractive species farther afield, or because increasing numbers of bees of Group A foraged on the experimental crop. Either or both factors may have been involved. Thus, Free (1959) found that with field beans the amount of bean pollen collected by colonies installed before flowering increased until it approached the amount collected by colonies moved to the crop after flowering began. But with opium poppies the amount of poppy pollen collected by colonies moved to the poppy field after it was in flower decreased during the experiment. Free and M. V. Smith (unpublished data) placed two colonies in a mixed pear orchard that was in full flower and found that the mean percentage of pear pollen out of the total pollen collected by the colonies steadily decreased from 85.4% in the first hour to 49.5% by the sixth hour.

A delay in taking colonies to crops until flowering has begun probably always increases pollination, particularly when the crop has a short flowering period or is less attractive to bees than other flowers in the locality.

ACKNOWLEDGMENTS.—We thank the following for their helpful cooperation during this work: G. F. Townsend and A. Adie of the Apiculture Department, Ontario Agricultural College; C. Black of the Sandhills' Research Station, North Carolina; W. A. Stephens of North Carolina State College; and G. H. Dickson of Vineland Experimental Station, Ontario. The senior author is indebted to the National Research Council of Canada for providing a fellowship during the tenure of which this work was undertaken.

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The Bean Leaf Skeletonizer, *Autoplusia egena*, and Its Control on Bush Snap Beans in the Everglades[1]

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ABSTRACT

Autoplusia egena (Guen.) infestations occurred in the Everglades in the spring of 1957, 1958, and 1959. The mean time required from egg to adult approximated 35 days. The parasite genera *Apanteles*, *Meteorus*, and *Copidosoma*, and predatory Pentatomidae *Podisus* spp. attacked the larvae. Of the materials tested toxaphene, dieldrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), Kepone® (1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one), Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane), SD-4402 (1,3,4,5,6,7,8,8-Octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), and *Bacillus thuringiensis* gave best control.

Rather serious injury by lepidopterous larvae occurred on bush snap beans in the Everglades in the spring of 1957, 1958, and 1959. Inspections revealed that more than 98% of the population was comprised of a single species of semi-looper. Specimens were reared to the adult stage at the Everglades Experiment Station and the moths of the more common species tentatively determined as *Autoplusia egena* (Guen.), the bean leaf skeletonizer. Harold A. Denmark of the State Plant Board of Florida, subsequently confirmed the identification. The other species was determined by Denmark to be *Pseudoplusia includens* Wlk., a semi-looper that appears somewhat more common on southern peas (cowpeas) than on snap beans. Since *P. includens* composed such a small percentage of the population, the species will be mentioned only incidentally elsewhere in this paper. *A. egena* has appeared very scarce on southern peas. A single larva was observed in July on soybeans.

Larvae of the bean leaf skeletonizer have been observed on beans in the area during previous seasons, but appear to have become more abundant since 1953 and they were quite injurious in some plantings of bush snap beans and pole beans in 1957 (Genung 1957, 1959). The species was reported by Lange (1943) to have caused considerable damage to pole beans in the Salinas Valley California, and in Orange County, California, during the intervening years (Campbell 1953, 1956). California infestations have been reported principally in the fall. However, Lange (1945) reported one infestation occurring in July 1936. Populations in the Everglades to date have been recognized only in the spring and early summer, and have been reported by Genung (1957, 1959). Two infestations in commercial fields were heavier than those observed in experimental plantings. Records supplied by Kimball (1959) indicate that *A. egena* in Florida is found mainly in the southern and central part of the State. The chronological spread on these data is from March to December. These data are from material of several collections. Crumb (1957) gives the distribution as "California and Florida southward into South America." Thus, the species would appear to be tropical and subtropical in its distribution. Observations over the past few seasons indicate that this phalaenid may possibly

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become of greater economic importance in the Everglades. Aside from the species' spring occurrence, little is known of its seasonal abundance in Florida. Since it occurred in January in California (Campbell 1957) it is probably capable of surviving the winter in southern Florida.

DESCRIPTION OF DAMAGE.—Injury by *A. egena* appears most severe on the foliage, but light feeding was observed also on the developing pods. Young larvae chew small holes in the leaves but older larvae consume the entire leaf area except the tougher ribs, from which habit the descriptive common name of the species is derived. Lange (1945) reports that pole bean varieties are the most severely attacked in California.

LIFE HISTORY.—Limited observations have been made on the biology and ecology of the species under Everglades conditions. Oviposition habits are similar to the cabbage looper, *Trichoplusia ni* (Hbn.). Eggs are laid singly or sometimes two or three together on the foliage. About 5 days are required for incubation during May. The larva is a typical semi-looper about the size of the cabbage looper, and is green to yellowish green, with paler dorsal and lateral stripes. The body has hairlike organs and setae that are more conspicuous than those on the cabbage looper. The larvae required 16 to 20 days to become full-fed prepupae during May and June. Pupation occurs in a silken cocoon within a leaf fold, and the pupal stage required a 9- to 12-day period during May and June. According to Lange (1945) several generations may occur in California. Observations in the Everglades to the present time have indicated that if more than two or three generations occur these must be under extremely low population levels. The adult moths are a grayish brown with lighter and darker markings on the forewings, and they approximate the cabbage looper in size. When flushed during daylight, the moths fly a few feet and quickly conceal themselves in the foliage.

PARASITES AND PREDATORS.—By late May 1957 considerable parasitism had occurred in the field. Larval parasites of two species were reared. Adult wasps of the parasites affecting *A. egena* were determined by C. F. W. Muesebeck to be *Meteorus autographae* Mues. and *Apanteles autographae* Mues. (Hymenoptera: Braconidae). Both species are known parasites of related Phalaenidae including the cabbage looper. The predatory Pentatomidae, *Podisus mucronatus* Uhler and *P. maculiventris* (Say), were the most important insect predators observed feeding on these larvae. A ground beetle *Caleida decora* (Fab.), a small foliage inhabiting form, was observed attacking the earlier instars.

In late May and early June 1958 larvae of both *A. egena* and *P. includens* were heavily attacked by the Polyembryonic encyrtid *Copidosoma truncatellum* (Dalms). The braconid *Apanteles autographae* was also very active in populations of *A. egena* during June 1958.

Maynards redwinged blackbird, *Agelaius phoeniceus floridanus* (Maynard), the boat tailed grackle, *Cassidex mexicanus westoni* Sprunt, and the Florida grackle, *Quiscalus quiscula quiscula* (L.), were also observed feeding on these larvae. Analyses of several stomach (gizzard) contents showed *A. egena* present in each instance in redwings.

Polyhedrosis disease was not observed affecting the species in the field, and attempts to induce infection with a cabbage looper virus, diagnosed by Dr. S. R. Dutky, were not successful in the insectary. An undiagnosed fungus disease, probably *Spicaria rileyi* (based on earlier diagnosis by Dr. Dutky), killed a few larvae in late May and June of each year. The many natural enemies of this insect appear to be of importance in its early summer disappearance in the Everglades.

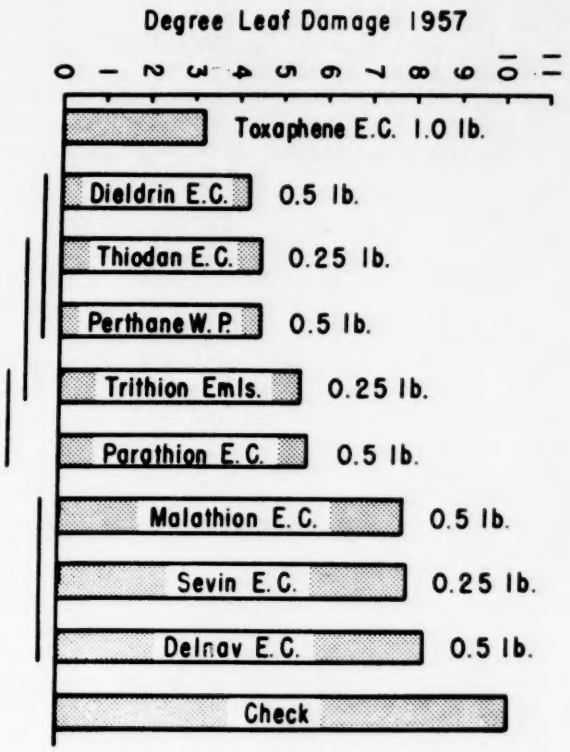
Degree Leaf Damage 1957
0 – 2 3 4 5 6 7 8 9 10 11
Toxaphene E.C. 1.0 lb.
Dieldrin E.C. 0.5 lb.
Thiodan E.C. 0.25 lb.
Perthane W.P. 0.5 lb.
Trithion Emls. 0.25 lb.
Parathion E.C. 0.5 lb.
Malathion E.C. 0.5 lb.
Sevin E.C. 0.25 lb.
Delnav E.C. 0.5 lb.
Check

FIG. 1.—Control of bean leaf skeletonizer 1957, based on square roots of number of moderately to seriously damaged leaves on two center rows of each plot. Means underscored by a common line are not significantly different. Others are significantly different.

1957 TEST.—Larvae of *A. egena* were the only injurious insects that appeared on bush snap beans in trials in sufficient infestation to obtain data on control in 1957. Seminole variety snap bean was planted as the test crop in a randomized complete block design experiment. Nine insecticides were compared with the untreated check. Treatments were replicated five times. Plots were 25 feet long and four rows wide with a row spacing of 3 feet. There was a 12-foot buffer area between plots and between blocks. Each material was applied as a spray at 200 p.s.i. One hundred gallons of spray were applied per acre. The insecticides used, formulations, and amounts of technical material applied per acre are shown in figure 1.

Evaluation of control was based on the amount of leaf damage by the larvae after the third weekly application. The number of trifoliate leaves having moderate to severe chewing injury was noted on all plants on the two center rows of each plot. Square roots of the recorded numbers were taken for statistical analysis.

All insecticide treatments were superior to the untreated checks. Toxaphene e.c. was significantly superior

to all other materials tested. Dieldrin e.c., Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) e. c., and Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane) w. p. also gave good control. Dieldrin was significantly better than the other two, which were not statistically distinct from each other. Trithion® (*S*-(*p*-chlorophenylthio)-methyl *O,O*-diethyl phosphorodithioate) e. c. and parathion e. c. gave fair control. Differences between the better treatments and the check appeared more striking in the field than were indicated by the counts. Malathion, Sevin® (1-naphthyl-*N*-methyl carbamate) and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) in the amounts used and under conditions of this test gave inadequate control of the bean leaf skeletonizer. None of the insecticides used caused any visible phytotoxicity on the plants nor resulted in yield reduction. The detailed results of this trial are shown figure 1. The multiple range test of Duncan (1953) also discussed by Le Clerg (1957) was applied in statistical treatment of the data.

1958 TEST.—Five insecticides were compared for skeletonizer control in a randomized complete block design experiment using five replications of each treatment. Tendergreen variety snap bean was used as the test crop. This variety became very heavily infected with rust *Uromyces phaseoli:* var: *typica* Art. and had been about 60% defoliated when treatment was commenced. Plot size and buffer zones were identical with the 1957 test. Treatments were made after an infestation of the larvae of *A. egena* appeared. A single application of the materials was made to an established infestation of moderate density. Mortality data were taken 48 hours after treatment. Insecticides were applied with a power sprayer at 200 lbs. p.s.i. delivering 98 to 100 gallons of spray per acre. The insecticide formulations and amounts of technical material applied per acre are shown in figure 2. Control was evaluated by counting the number of surviving larvae on 100 plants per plot.

Fewer surviving larvae were counted in the toxaphene e. c. treatment. Thiodan e. c., Phosdrin e. c., and Kepone® (1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$]decan-4-one) w. p. had but slightly more surviving larvae than toxaphene and were not significantly inferior thereto. Phosphamidon was significantly superior to the check but inferior to all other treatments except Kepone. The results of this trial are shown in figure 2. Since *P. includens* composed such a small percentage of the population, the control obtained is considered essentially that of the bean leaf skeletonizer. Phytotoxicity was not observed in any of the treatments. Statistical methods used were identical with the 1957 test.

1959 TEST.—Eight chemical insecticides were compared with *Bacillus thuringiensis* Berliner and an untreated check in the spring of 1959. Plot size, design, gallonage, pressure, and equipment were similar to those used in the 1957 test. The insecticide formulations and amounts of technical materials applied per acre are shown in figure 3. Control was evaluated by counting the number of surviving larvae on 20 plants from the middle rows of the plots.

All insecticide treatments were superior to the check. While SD-4402 (1,3,4,5,6,7,8,8-Octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) indicated perfect control, it was not significantly superior to Kepone, toxaphene, *Bacillus thurinigiensis*, or Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate). These treatments were significantly better than Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) and dimethoate, which were not statistically distinct. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) gave relatively poor control and Sevin, which was significantly better than Diazinon, gave only fair control. Figure 3 shows the comparative effectiveness of the various treatments. Statistical procedures were the same as those used for the previous seasons.

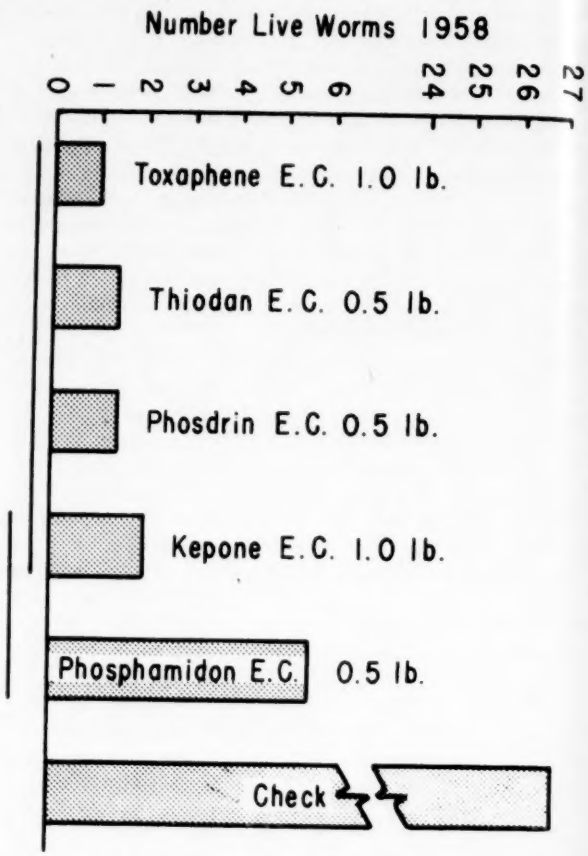
Number Live Worms 1958
0 1 2 3 4 5 6 24 25 26 27
Toxaphene E.C. 1.0 lb.
Thiodan E.C. 0.5 lb.
Phosdrin E.C. 0.5 lb.
Kepone E.C. 1.0 lb.
Phosphamidon E.C. 0.5 lb.
Check

FIG. 2.—Control of bean leaf skeletonizer 1958, based on number of surviving larvae per 100 plants per plot. Means underscored by a common line are not significantly different. Others are significantly different.

DISCUSSION AND CONCLUSIONS.—Since the bean leaf skeletonizer *A. egena* has become increasingly injurious to snap beans and pole beans in the Everglades in recent years, the species would appear to have a considerable economic potential. The relatively serious damage of 1957, 1958, and 1959 was particularly noticeable. Trials conducted for three consecutive seasons show that certain insecticides that are available and registered for use on beans are effective for control of this species. Several experimental materials also show promise. Toxaphene was outstanding for three seasons, while Phosdrin and Kepone performed well for the two seasons they were

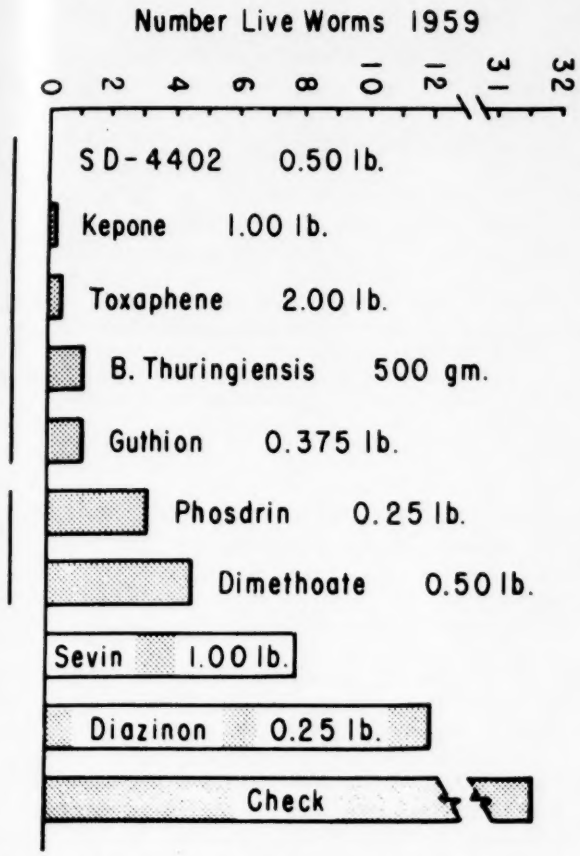

FIG. 3.—Control of bean leaf skeletonizer 1959, based on number of surviving larvae per 20 plants per two center rows per plot. Means underscored by a common line are not significantly different. Others are significantly different.

tried. Thiodan and SD-4402, Guthion, dieldrin, and Perthane were outstanding during the single seasons they were tested. *Bacillus thuringiensis* during the one season it was included was not significantly inferior to any chemical insecticide tried. All the remaining materials were only fair to poor under conditions of these tests. No definite reason for the heavier skeletonizer populations of recent seasons is known. Temporary imbalances either directly favorable to the phalaenid or inimical to some of its enemies may have prevailed. During the 3-year period of these observations the species has been heavily attacked by parasites and predators, and these have appeared to be important in regard to its disappearance in the late spring and early summer. While the species will probably subside to its former inconsequential status, the possibility remains that this leaf skeletonizer could become either a seasonal or sporadic pest of beans in south Florida. The literature indicates that in California the species, while capable of causing considerable injury, is sporadic in its economic appearances.

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BOOK REVIEW

CHOLINESTERASES: A HISTOCHEMICAL CONTRIBUTION TO THE SOLUTION OF SOME FUNCTIONAL PROBLEMS, by M. A. GEREBTZOFF (1959) Pergamon Press, N. Y., 195 pp. $8.50.

Histochemistry holds a central position at the convergence of morphological, physiological, and biochemical research. However, few entomologists have used the tools for precise localization of substances. Over 4,000 articles have been written on cholinesterase; nearly 400 are cited in this book; of these, only four utilized insects. Organophosphorus insecticides are not even mentioned.

"Cholinesterases" represents 6 years of team work in the Department of Anatomy Laboratory of Liege University. Gerebtzoff's research goal apparently is to locate and distinguish esterases in the vertebrates with all possible precision and then to explain why they are there. The chapters are arranged by organ system, and the subject index refers principally to tissues and not to ideas, problems, or methods. The wording is pleasingly free of pompous phrases. The style jolts the reader into thinking through the problems presented and emphasizes the limitations and possible artifacts of applicable techniques. For example, differentiating the origin and the location of enzymes or metabolites merits contemplation. The book is profusely illustrated with 125 photomicrographs and seven plates.

"Cholinesterases" should be inspirational reading for the insect physiologist who realizes that working on brei sometimes can be misleading and for the morphologist who would like to see molecules. Most of us should scan this short monograph to gain more appreciation of methodology, but it isn't a reference book for entomologists. This monograph deserves emulation on invertebrates with cholinesterases and with other enzymes.

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Effects of Low Temperature on the Survival of the Alfalfa Weevil from Alberta and Utah[1]

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Abstract

Alfalfa weevils, *Hypera postica* (Gyll.), were collected from Alberta and Utah in the spring of 1957. Their progenies were reared for one generation under indentical conditions in the laboratory and the adults of the two populations were compared for mortality at low temperatures. The two groups did not differ in their undercooling points, which averaged about −18° C.

After 72 days of exposure to −10° C. there was no difference in mortality between the Alberta and Utah weevils. However, when moved to 10° C. after 11 days at −10° C. the Utah weevils became active much sooner than the Alberta weevils.

Alberta and Utah weevils were compared for mortality in the field during the winter of 1957–58 in southern Alberta. In one experiment the weevils were allowed to select their own habitat. In a second experiment they were restricted to four positions. In both experiments there were no significant differences in mortality between the two groups of weevils. It was concluded that the establishment of the alfalfa weevil in more northerly regions did not arise through development of increased cold resistance but, perhaps, through a selection of those individuals that possess slower responses to rising temperatures.

The alfalfa weevil, *Hypera postica* (Gyll.), was recorded from alfalfa in Utah in 1904 by Titus (1910). This record was the first for the weevil in North America.

Cook (1925) attempted to determine the northern limits of the economic distribution of the alfalfa weevil in the United States from its distribution in various ecological regions in Eurasia. He suggested that the northern range of the weevil was limited by its inability to survive the colder winters. His predictions held true for about 12 years. Then 15 years later the weevil was reported by Hamlin *et al.* (1949) established in southern Montana. Apparently it confined its activities to the known infested areas for another 14 years before it was reported from northern Montana by Pepper (1953) and from the Eastern United States by Poos & Bissell (1953). In 1954 (Hobbs *et al.* 1959) it was discovered in southeastern Alberta in Canada.

The northern extension of the insect's distribution suggested that it may have become adapted to survive these lower temperatures. Therefore, the cold resistance of weevils from Utah and from southern Alberta was compared in the field and in the laboratory. The study was limited to the effects of cold on the overwintering adult weevils.

Methods and Materials.—*Rearing.*—In the late spring of 1957, eighty adult weevils were collected in Utah[3] and 100 were obtained from the Milk River area of Alberta. They were increased by rearing them under identical conditions in the laboratory for one generation.

The weevils were put in small celluloid cages over greenhouse-grown alfalfa plants and left at 25° C. until larvae appeared. The larvae were removed from the plants and placed in glass stacking dishes 7 inches in diameter and 3 inches high. The adults were re-caged over new plants. The larvae in the stacking dishes were kept provided with newly cut alfalfa until they pupated and emerged as adults. Twelve hundred adult Utah weevils and 1,500 adult Alberta weevils were obtained.

As the adults emerged they were removed daily and placed with newly cut alfalfa in 2-quart jars with screen tops. The cuttings were replaced every 2 days. After the weevils stopped feeding they were acclimated by being subjected to 20°, 15°, and 10° C., successively, 7 days at each temperature. Fresh cuttings were supplied at intervals to maintain a relatively high humidity. Then they were placed at 5° C. and periodically supplied with fresh alfalfa cuttings until 1 week before they were to be used. During that week they were kept without food at 80% relative humidity to insure that they would have no food in their guts.

Laboratory Experiments.—*Undercooling points.*—Sixty adults taken at random from each stock were left at room temperature for 2 hours so that any moisture condensed on their bodies would evaporate (Salt 1936). Then 25 were chosen for uniformity in size from each stock. Single weevils from the two stocks were undercooled alternately by the methods used by Salt (1953). At the end of the experiment the weevils were dissected and sexed.

Effects of Long Exposure to −10° C.—A temperature of −10° C. was selected because it was slightly above the undercooling points determined by the previous test. Blocks 1×4 inches, 7 inches long, were each drilled with 50 holes $\frac{3}{8}$-inch in diameter and $\frac{3}{8}$-inch deep, spaced $\frac{5}{8}$-inch apart. One weevil was placed in each hole. Each block contained either Alberta or Utah weevils. Each treatment was replicated four times. Paired samples were placed on wire racks in a 3-gallon crock at −10° C. The crock was aluminum-foil covered and contained cracked ice to maintain a high relative humidity. The positions of the samples in the crocks were randomized.

The first treatment was designed to determine the progressive mortality. The crocks were moved to 5° C. for 5 hours every second day for 26 days, then once every fourth day for the next 28 days. The percentage mortality for each stock was determined each time before being re-

[1] Portion of a thesis submitted to the graduate school North Dakota Agricultural College, Fargo, in partial fulfillment of the requirements for the degree of Master of Science. Contribution from the Entomology Section, Canada Agriculture Research Station, Lethbridge, Alberta, Accepted for publication January 25, 1960.

[2] The author expresses his appreciation of the advice and criticism received during the progress of the work and the preparation of this paper from Drs. N. S. Church, G. A. Hobbs, N. D. Holmes, R. W. Salt, G. E. Swailes, and Mr. L. A. Jacobson, all of the Canada Agriculture Research Station, Lethbridge, Alberta, Dr. C. W. Farstad, formerly Head of Entomology Section, Research Station, Lethbridge, now Director of Plant Protection Division, Production and Marketing Branch, Canada Department of Agriculture, Ottawa, and Dr. J. A. Callenbach, North Dakota Agricultural College, Fargo, under whose guidance the thesis was presented.

[3] Supplied by Dr. G. F. Knowlton, Extension Entomologist, Utah State University, Logan.

turned to −10° C. Weevils that did not move when touched were recorded as dead.

In the second treatment the weevils were left at −10° C. without interruption until the mortality of the Alberta stock in the first treatment reached approximately 50%. Then they were moved to 5° C. for 6 hours and the mortality was recorded.

Recovery at 10° C. After Storage at −10° C. for 11 Days.—The weevils used were those that survived the two treatments described above.

These insects were stored for 3 days at 15° C. to remove most of the chilling effects of their exposure to −10° C., after which they were returned to −10° C. for an additional 11 days. They were then placed at 10° C., and the time at which each individual began to crawl was recorded continuously for 12 hours, then at 24-hour intervals for the next 96 hours.

FIELD EXPERIMENTS.—*Experiment 1.*—The adult weevils were obtained from the bulk supply stored at 5° C. They were placed at 10° C. and 80% relative humidity until conditions in the field were favorable for their release.

On October 31, 1957, 12 galvanized cylindrical cages were placed in pairs over separate alfalfa plants in a plot near the Lethbridge laboratory. The cages were each 8 inches in diameter and 4 inches high and were forced about ½ inch into the soil. They were set out in a line at right angles to the prevailing westerly wind so that the snow would drift on all cages equally. Ten Alberta weevils were dropped into one cage of each pair and 10 Utah weevils into the other. The cages were covered with 20-mesh plastic screen so that the insects would not crawl out before they hibernated. The tops were removed when the temperature dropped to the freezing point 12 days later. The cages immediately started to fill with snow and within 2 days they were covered.

The weevils were left in the cages until March 18, 1958. From each cage the plants, litter, and the soil to a depth of 1 inch were brought into the laboratory, where the material was carefully examined for weevils. The percentages of dead weevils from both groups were converted by the angular transformation and analyzed by Student's "t" test method (Cox 1954).

Experiment 2.—Also on October 31, 1957, a considerable number of weevils were placed in cylindrical cages and buried at various depths in litter and soil. The cages were made of 24-mesh bronze screen and were 3¾ inches long and 1 inch in diameter. They were half filled with a mixture of soil and alfalfa litter and corked at each end. Two cages were placed together as paired samples, one containing six Alberta weevils and the other six Utah weevils. The cages were placed in four positions along the east side of a row in the alfalfa plot. The positions were: horizontal and half buried in the soil so that the soil and litter in the cage were level with the surface of the soil; horizontal on the surface of the soil but covered with up to an inch of alfalfa litter; horizontal and buried ½ inch below the surface of the soil; vertical and half buried in the soil. The treatments were randomized and replicated eight times.

On March 6, 1958, the cages were taken up and the number of weevils that had died in each cage was recorded.

Table 1.—Cumulative mortalities of Alberta and Utah weevils at −10° C.

Days at −10° C.	Percentage Mortality	
	Alberta weevils	Utah weevils
1	14.0	12.5
8	20.0	20.5
14	23.5	22.0
20	26.5	25.5
26	37.5	30.5
34	51.5	38.5
42	61.5	43.5
50	63.0	47.0
64	78.0	70.0
72	86.0	81.0

RESULTS.—LABORATORY EXPERIMENTS.—*Undercooling Points.*—Both Alberta and Utah weevils had undercooling points that ranged from −13.5° to −23.5° C. They averaged −18.8° and −18.1° C., respectively. The Alberta and Utah weevils did not differ significantly in their resistance to freezing. The undercooling points of male and female weevils were in the same range.

Effects of Long Exposure to −10° C.—In the first exposure treatment (table 1) a high mortality in both Alberta and Utah weevils occurred during the first 24 hours. This percentage probably comprised those weevils that had undercooling points near −13° C. For the next 71 days mortality occurred gradually in both groups. A slope function ratio (Litchfield 1949) obtained from the data in table 1 showed that the two mortality curves were parallel and that no significant difference occurred between the two groups of weevils.

In the second exposure treatment the weevils were removed from −10° C. after 34 days. The mean mortalities of the Alberta and Utah weevils after 34 days at −10° C. were 31.3 and 26.0. These means did not differ significantly.

Recovery at 10° C. After Storage at −10° C. for 11 Days.—Table 2 shows that the Utah weevils became active much more rapidly than did the Alberta weevils at 10° C. after storage at −10° C. Within 6 hours of warming more than twice as many Utah weevils were active as Alberta ones.

FIELD EXPERIMENTS.—*Experiment 1.*—The mean mortalities of the Alberta and Utah weevils caged overwinter on alfalfa plants were 2.3 and 2.8 respectively. The means did not differ significantly, indicating that the Alberta and Utah weevils were equally able to survive in the

Table 2.—Percentages of Alberta and Utah weevils that crawled at various times at 10° C. after being stored for 11 days at −10° C.

Group	No. Weevils	Hours					
		6	12	24	48	72	96
		Series 1					
Alberta	21	14	38	48	72	86	100
Utah	28	39	61	75	93	100	100
		Series 2					
Alberta	27	26	37	52	78	85	100
Utah	54	50	74	76	98	100	100

Table 3.—The mean mortalities per cage of six weevils for the four positions in both groups in the field.

	Positions of Paired Cages			
	Completely Buried	Half-Buried		On Soil Surface with Litter
		Horizontal	Vertical	
Mean mortality per cage	1.63	1.06	2.00	3.37

L.S.D. at 1% level 1.15

cages under the field conditions of the winter of 1957–58.

Experiment 2.—In the second field experiment both groups of weevils survived the winter equally well. An analysis of variance between groups showed no significant difference between the Alberta and Utah weevils when all the positions were considered.

Table 3 shows the mean mortalities per cage of six weevils for the four positions in both groups in the field.

The mortality of the weevils on the soil surface and covered lightly with alfalfa litter was significantly higher at the 1% level than that for the other three positions. The relatively high rates of survival in the other more protected positions agree with Titus' (1910) and Milliron's (1956) findings that most weevils would live if protected by litter and soil.

Discussion.—Cook (1925) believed that low winter temperatures limited the successful spread of the weevil. However, the laboratory and field experiments conducted herein showed that the Alberta weevils had no greater resistance than the Utah weevils to low winter temperatures. Hence, there must be some other explanation for the establishment of the alfalfa weevil in Western Canada.

During the winter in southern Alberta severe cold periods are often followed by mild weather; records from the Meteorological Observing Station at Lethbridge show that chinook winds have raised the temperature from −25° to 45° F. within a 24-hour period. The protective snow cover is often removed completely, exposing hibernating weevils to the severe temperatures that often follow.

Poos (1955) observed that more adults of the corn flea beetle, *Chaetocnema pulicaria* Melsh., survived a uniformly cold winter than one having several mild periods followed by rapid changes to much lower temperatures. Chittenden (1896) in Massachusetts reported that large numbers of hibernating asparagus beetles, *Crioceris asparagi* (L.), were killed in winter during severe cold periods, after periods of mild weather.

Reeves *et al.* (1916) and Sweetman (1929) found that adult alfalfa weevils become active in the winter when the temperature rises above freezing. Weevils that move from their hibernation sites during the mild weather caused by a chinook might then be trapped in exposed positions when the temperature subsides again. Furthermore, weevils have been observed to drink and feed shortly after becoming active. Salt (1953) has shown that food in the gut of an insect inoculated freezing in its tissues. Hence, material ingested by the weevils may make them more susceptible to freezing during any subsequent rapid drop in temperature. Thus the slower activation of the Alberta weevils when the temperature is raised may be an important factor in the establishment of weevils in Alberta, because it would tend to prevent them from leaving their hibernation sites and from drinking.

The winter of 1957–58 in southern Alberta was unusually mild; the minimum temperatures were generally above −10° C. except during March, when they were as low as −30° C. Hence, the weevils in the field experiments were not exposed to violent temperature fluctuations and the differential mortalities that might have been expected did not occur that season.

The observation that Alberta weevils became active more slowly than those from Utah when the temperature is raised may help to explain why, in relation to the growth of the alfalfa plant, the weevils in Alberta become active later in the spring than they do in Utah. In Alberta, Hobbs *et al.* (1959) reported that few weevils were found on the alfalfa plants until they were about 11 inches high, whereas Hamlin *et al.* (1949) stated that in Utah the weevils began laying eggs in the litter and broken alfalfa stems even before the alfalfa plants started to grow.

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Preliminary Evidence of Resistance of High-Amylose Corn to the Angoumois Grain Moth[1]

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ABSTRACT

Angoumois grain moths (*Sitotroga cerealella* (Oliv.)) were reared on corn in pint jars in the laboratory. Ears of corn containing from 0% to 75% amylose[4] were used. Moths placed on the high-amylose kernels produced larvae that developed more slowly, fewer moths reached the adult stage, and the adults weighed less than the moths from normal corn, which has an amylose content of about 27%. The possibility of employing this insect for separating kernels with different amylose contents is discussed.

The Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) is the most important pest of stored ear corn in Missouri and is one of the most commonly found grain-infesting moths in the United States. It is one of the many insect pests for which host-plant resistance would be the most desirable method of control. Warren (1956) found only slight differences in a study of 29 single-cross hybrids. The present study was initiated after a fortuitous observation of differences in moth-infestation levels among ears of corn being segregated for normal and high-amylose content.

High-amylose corn is a recent development (Zuber *et al.* 1958) and has considerable industrial potential according to Dimler (1951). The amylose molecule is a linear component of cornstarch while amylopectin has a branched type molecular structure. Starch in commercial corn is about 27% amylose and 73% amylopectin. The amylose content of the corn used in these studies varied from 0% to 75%.

MATERIALS and METHODS.—A preliminary test was conducted comparing four ears of high-amylose corn with ear portions of U. S. 523W and Kan. 1639, which have normal amylose content. Tests of the high-amylose ears indicated a range from 67% to 75% amylose. Each ear portion was placed in a pint carton and infested with 40 adult Angoumois grain moths. The cartons and jars of subsequent tests were kept in an insect-rearing room in which some control over temperature and humidity was possible. Counts of emerging moths were begun 34 days later and made at 2- to 6-day intervals until 100 days after infestation. During the first test, the average temperature was 73° F. with a high of 95° and a low of 58° for the 100-day period. The weekly averages ranged from 69.8° to 76.3°. The emerging moths were counted and removed by aspirators connected to a vacuum cleaner for a constant suction source.

Another comparison of ear portions ranging in amylose content from 0% to 54% was begun 2 months after the first test. No moths emerged from the ears of below-normal amylose content at the first infestation. Ears with average or high amylose percentages yielded results similar to those of the first tests.

In the third test, corn ears with known amylose contents were shelled and 100 kernels placed in wide-mouth pint jars with 60-mesh screen replacing the lids. The screens were held in place by the jar rings. As the ears were variable in size, the number of replicates per ear varied from one to four. Each jar was infested with 20 adults and the adults removed after 7 days. The first moths emerged on the 33rd day after infestation. Daily counts were made for the next 40 days, except on Sundays. Average temperature for the period was 77° F. The weight and sex of all moths were recorded by jar and date. Because a few moths escaped, more moths are included in the emergence counts than in the weight comparisons. The data were analyzed by using the "F" and "D" tests as described by Snedecor (1956) to determine significant mean differences.

Table 1.—Moth emergence from eight normal and eight high-amylose corn ear portions at intervals after infestation.

Amylose Level	Moths Emerging During Indicated Days After Infestation					
	34–40	41–45	46–50	51–60	61–80	81–100
Normal	215	441	249	207	141	125
High	1	3	6	14	37	152

RESULTS and DISCUSSION.—The numbers of moths emerging from the first test are shown in table 1. The average number of moths emerging from each of the 8 jars with normal ear portions reached a peak of 12 moths per day on the 42nd day and by the end of the test most normal kernels had 1 or more emergence holes. The number of moths that emerged from the high-amylose ears increased slowly for the entire period indicating that the larvae could survive, but that development was greatly retarded. The high-amylose portions were retained another 100 days, but the emergence rate decreased and the population could not maintain itself. Adverse temperatures or humidity conditions may also have affected this latter fact.

The results of the second test were similar to those of the first, in that ears containing 35%, 45%, and 53.7% amylose all appeared to retard development. Ears with 0% and 2.5% amylose had an equally deleterious effect.

The results of the third test are summarized in table 2 for the different factors evaluated. The average number of moths emerging per jar can be considered roughly as the per cent infested kernels, since each jar contained 100 kernels. Probably because of the small sample size, only the two highest and three lowest emergence rates were

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[4] The corn with the high-amylose starch used in this study was obtained from the Cooperative Research Project between the Field Crops Department of the University of Missouri; the U. S. Department of Agriculture, Crops Research Division; and the U. S. Department of Agriculture, Northern Utilization Research and Development Division.

Table 2.—Comparative responses of the Angoumois grain moth when placed on corn samples varying in amylose content.

Amylose Content (%)	No. of Jars	Average No. of Moths Emerging	Average Time of Emergence (Days)	Average Weight of Males (Mg.)	Average Weight of Females (Mg.)
1.0	4	40	48	3.89	6.33
25.6	3	46	41	4.18	6.74
35.0	1	24	52	3.61	5.94
47.2	2	26	48	2.54	6.13
51.5	4	13	49	3.44	5.46
56.1	3	11	55	3.19	5.93
63.8	3	12	62	3.35	5.35
69.9	2	45	50	3.65	5.79
			D	0.52	0.75

significantly different from one another.

The average time of emergence was calculated by multiplying the number of moths emerging by the number of days since infestation. Moths from normal or 25.6% amylose corn emerged first and were the first to reach a peak of emergence. These data indicate some association between increased amylose content and an increase in the larval development period even though moths from the 69.9% amylose ear did not entirely follow this pattern.

A total of 275 female and 264 male moths from the various jars was weighed. A few significant differences occurred in weights of females or males from replicates of the same ear, but results from most of the ears indicated but little within sample variability. It should be emphasized that the amylose content was determined from kernels removed from the ears prior to exposure to the moths. The remaining kernels used in this test were assumed to be similar in amylose content. However, this material had not been inbred to the extent of reaching complete homozygosity. Therefore, the possible range in amylose content of individual kernels might influence these results.

Further investigations should be made to determine the nature of this apparent case of host-plant resistance, The resistance expressed suggests the antibiosis type, since larval development was prolonged, fewer moths reached adulthood, and the resulting adults were lighter in weight.

The possibility of other factors associated with high-amylose content should not be overlooked. Some recent studies at the Missouri Agricultural Experiment Station indicate that high-amylose kernels have a harder texture, and also have higher endosperm protein and endosperm oil contents (Zuber *et al.* 1959).

The use of the Angoumois grain moth for screening kernels ranging in amylose content might provide a very desirable technique in breeding high-amylose corn. This preliminary study plus other observations indicate that this insect will destroy the viability of normal kernels before destroying the viability of the high-amylose kernels. If this insect would select a 35% amylose kernel in preference to a 70% amylose kernel, this method could be used in examining large numbers of kernels varying in amylose content. At a certain point the activity of the larvae could be stopped by freezing or fumigation, leaving the high-amylose kernels for production of the next generation of corn. Current studies are now underway to determine whether this insect can be used in this manner. This technique would be of great benefit, since the current chemical determination requires at least 10 kernels, and progenies cannot be grown from kernels used for analysis.

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INDEX XVI

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The Effect of Seed Treatments with Phorate and Other Systemic Insecticides on the Germination of Wheat[1]

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Abstract

Studies were conducted in the greenhouse to determine the relative effect of phorate, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate), and SD-3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate) on the germination of wheat when applied as a seed treatment. Extensive testing of phorate was carried out in a factorial experiment using rates from 0.18 to 1.5 pounds of active toxicant per 100 pounds of seed, with six fungicides and five stickers.

Both phorate and SD-3562 were seriously toxic to wheat seed, with the latter being most toxic under the test conditions. Di-Syston did not significantly reduce seed viability with rates as high as 1.5 pounds per 100 pounds of seed. The data suggest interactions between certain stickers and fungicides to reduce phytotoxicity of phorate. Another interaction between phorate and a sticker composed of 3 parts dextrin, 1.5 parts sorbitol, and 0.5 part water to lessen the toxic effect of the insecticide to the seed was apparent.

Phytotoxic effects of phorate on seed viability increased as the interval between treatment and seeding was prolonged. However, data indicate that this was largely due to the sticker used to apply the phorate. This effect was slight with the sticker mentioned above, but very severe with another sticker composed of 1.4 parts rice oil, 2 parts sorbitol, and 0.6 part dextrin. Phytotoxic effects are apparently reduced by the interaction of phorate with the fungicide and sticker. The adverse effects of phorate when applied as a seed treatment to wheat at the rates most likely to be used in the field, and with the use of a suitable sticker and fungicide, should be greatly reduced.

Control of the hessian fly (*Phytophaga destructor* (Say)) has been accomplished in the past by cultural means, particularly delayed seeding and the use of resistant varieties. However, with an increasing need for fall forage in many areas, there may be a trend toward earlier seeding of susceptible wheat. Under these circumstances, the crop may need to be protected against insects, particularly the fall generation of the hessian fly. The use of a systemic insecticide might enable the farmer to seed wheat several weeks earlier without being limited to the few existing resistant varieties now available. Some of the newer materials show promise as a means for control of insects when used as a seed treatment.

Brown (1957) first reported control of the hessian fly with systemic organic phosphates applied in a mixture with fertilizer in the drill row. Guyer *et al.* (1958) reported the results of field tests using phorate and Di-Syston® (*0,0*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) as seed treatments for control of hessian fly. They reported superior control with phorate than with Di-Syston. However, phorate adversely affected germination. Wilson *et al.* (1958) reported reduced stands from the use of phorate as a seed treatment in Indiana. Gifford *et al.* (1959) tested the effects of phorate on germination and seedling survival of three varieties of wheat using soybean, peanut, corn, and rice oils, and water as stickers. They found that germination was not adversely affected by dosages of phorate as high as 1 pound per 100 pounds of seed when water was used as the sticker, but that seedling survival was reduced by dosages above 0.75 lb. They also found that germination was reduced and seedling survival was lower when oils were used.

In 1958 studies were initiated at Purdue University to find a combination of sticker and fungicide which, when used with phorate in a seed treatment, would not impair germination. Studies were also undertaken to determine if the time interval between treatment and planting of the seed had an influence on germination.

Methods and Materials.—All experiments were carried out in the greenhouse using the variety, LaPorte. Phorate (Thimet 44-D) was applied to the seed at the rates of 0.18, 0.37, 0.75, and 1.5 lbs. of actual toxicant per 100 lbs. of seed. In combination with these dosages, the fungicides arasan, captan, thiram, panogen, Dow 9-B, and Dow 9-B liquid were tested at rates of 3 and 6 oz. per 100 lbs. of seed.

Several stickers, which will be referred to by letter in the tables, were tested as follows: A) solution of 1.4 parts rice oil, 2 parts sorbitol, and 0.6 part dextrin, B) solution of 3.0 parts dextrin, 1.5 parts sorbitol, and 0.5 part water, C) solution of 1.0 part aerotex resin e. u., 3 parts dextrin, 1 part arlex, D) solution of 0.5 part aerotex resin 159, 1.5 parts sorbitol, and 2 parts of water, E) solution of 1.5 parts aerotex resin 159, 1.5 parts polyethylene glycol 400, and 2 parts of water.

All combinations of phorate, fungicides, and stickers were used in a complete factorial study. All materials were applied to the seed by W. B. Gehl of the American Cyanamid Company.

In other studies, the effect of Di-Syston and SD-3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate) were tested. With these materials, no attempt was made to find a sticker-fungicide combination that would lessen phytotoxicity.

In all experiments, the treated seed was planted in flats filled with a 2 to 1 mixture of silt-loam and sand. Standard 20″ by 14″ flats were divided into seven rows running parallel with the short side of the flat. Each of these rows was then divided equally by a strip of aluminum foil inserted into the soil. This made 14 half-row plots, all of which were separated by aluminum foil barriers inserted into the soil so that translocation of chemicals could not take place between treatments. Treatments were replicated four times, each plot being seeded with 25 seeds. The wheat commenced to emerge in 3 days and there

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Table 1.—The influence of various combinations of stickers and fungicides with phorate on the per cent germination of wheat.

Fungicide	Sticker A	Sticker B	Sticker D	Sticker E	Fungicide Means
None	54.9	76.6	59.8	78.8	67.4
Arasan	55.2	82.0	79.6	77.5	74.7
Captan	55.4	80.1	84.5	76.8	74.9
Thiram	51.8	81.0	69.1	74.2	69.5
Sticker means	55.0	79.5	73.8	76.9	

L.S.D.	5%	1%
Fungicide means	2.1	3.6
Sticker means	2.1	3.6
Within fungicides	8.0	13.7
Within stickers	8.0	13.7

Table 2.—Per cent germination of wheat treated with phorate and various stickers.

Sticker	Pounds Phorate per 100 Lbs. Seed 0	0.18	0.37	0.75	Sticker Means
A	58.4	56.1	56.2	49.3	55.0
B	86.3	80.0	75.2	75.8	79.5
D	89.3	72.4	75.0	54.6	73.8
E	82.9	80.4	76.9	66.2	76.9
Phorate dosage means	80.3	72.7	71.1	61.8	

L.S.D.	5%	1%
Sticker means	2.1	3.6
Phorate dosage means	2.1	3.6
Within stickers	8.0	13.7
Within phorate dosages	8.0	13.7

was usually no significant increase after 7 days, at which time counts were made of growing seedlings.

Using the same technique, tests were conducted to determine the influence storage might have on the germination of treated seed. Tests were conducted at 5, 30, 60, and 90 days after treatment. Four stickers, A, B, D, and E were used with captan and 0, 0.25, and 0.50 pounds of phorate per 100 pounds of seed.

Results.—The analysis of variance in five experiments showed significance at the 1% level for differences between fungicides, between stickers, and between phorate rates. As the dosage of phorate was increased, the viability of the seed declined, although this was partially alleviated by the addition of a fungicide at the rate of 3 oz. per 100 lbs. of seed. Increasing the fungicide to 6 oz. did not in general increase germination.

Interactions which were highly significant occurred between phorate and stickers, phorate and fungicides, and between stickers and fungicides. Examination of the data also suggests that a three way interaction occurred between fungicides, stickers, and phorate. Preliminary studies showed that higher germination was obtained with arasan, captan, or thiram, than with panogen, Dow 9-B, or Dow 9-B liquid as fungicides. Of the stickers tested, sticker C (1.0 part aerotex resin e. u., 3 parts dextrin, 1 part arlex) was found to inhibit germination almost 100%.

The four stickers and three fungicides found to be superior in previous tests were retested together and the data are presented in tables 1 and 2. Higher germination was obtained with captan and arasan than with thiram throughout the overall range of treatments. Sticker B was significantly superior to the other stickers tested. Examination of the data (table 1) suggests that an interaction took place between stickers B and D and the fungicides to reduce phytotoxic effects of phorate, regardless of dosage. This interaction appears most pronounced between sticker D and arasan or captan. Another interaction (table 2) appears to have occurred between phorate and sticker B, lessening the toxic effect of the insecticide on the seed.

A comparison of the toxicity of phorate to wheat seed with Di-Syston and SD-3562 under comparable conditions is shown in table 3. In these tests, Di-Syston was not significantly toxic to the seed in contrast to phorate and SD-3562. The phytotoxicity of SD-3562 was apparently greater than phorate because no seeds germinated at a rate of 1.5 pounds per 100 pounds of seed. In contrast, with phorate at the same rate, 23% of the seeds germinated and developed normal seedlings.

The effect of storage on germination of seed treated with phorate is shown graphically in figure 1. Toxicity to the seed was more severe as storage time increased. However, this effect was least with sticker B (3 parts dextrin, 1.5 parts sorbitol, 0.5 part water). With sticker A (1.4 parts rice oil, 2 parts sorbitol, and 0.6 part dextrin) it was very severe.

Conclusions.—It is concluded from these studies that, although phorate when applied as a seed treatment to wheat reduced germination, this effect may be greatly reduced by using a fungicide such as captan or arasan and a suitable sticker. In these studies, the most effective sticker (B) consisted of 3 parts dextrin, 1.5 parts sorbitol, and 0.5 part of water.

Phytotoxic effects are apparently reduced by the interaction of phorate with the fungicide and sticker. It is further concluded that reduction in seed viability following storage of phorate treated seed is largely due to the sticker used.

Data from studies to be reported in another paper suggest that probably dosages to control insects on wheat will be in the range of 0.20 to 0.30 pound of active phorate per 100 pounds of seed. Using the fungicide-sticker com-

Table 3.—Per cent germination of wheat treated with phorate, Di-Syston, and SD-3562 in combination with captan at 3 oz./100 lbs. of seed

Rate of Toxicant per 100 Lbs. Seed	Per Cent Germination Phorate	Di-Syston	SD-3562
None	78.0	74.0	81.0
0.18	89.0	82.0	92.0
0.37	81.0	85.0	79.0
0.75	43.0	86.0	52.0
1.50	23.0	80.0	0.0
L.S.D. at 5%	4.9	n.s.	3.6
at 1%	6.9	n.s.	5.1

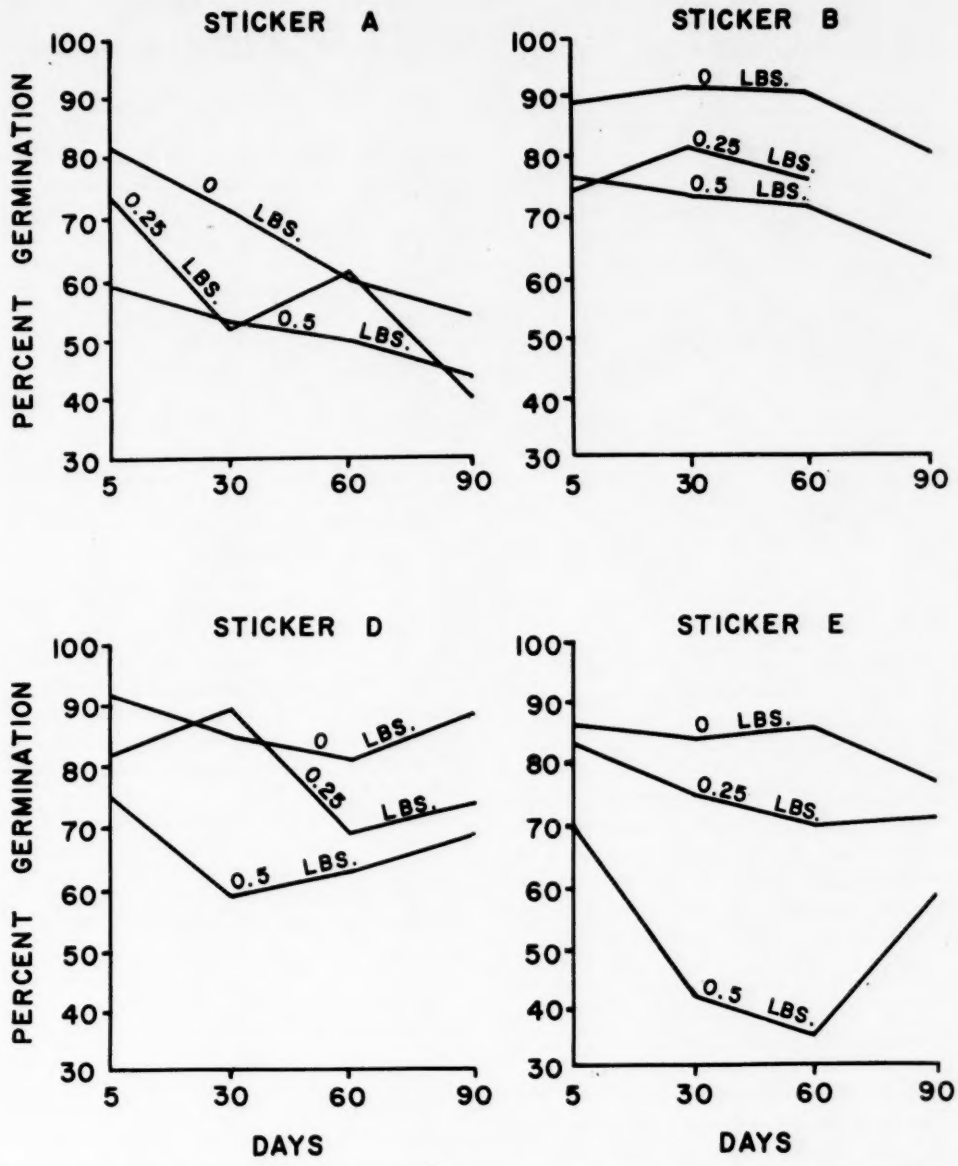

FIG. 1.—The effect of storage on the germination of wheat seed treated with phorate and various stickers.

bination suggested above, phytotoxicity should be minimized at these low dosages.

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Insects Associated with Wounds on Trees that Develop Oak Wilt[1]

W. D. Buchanan[2]

Abstract

In the 4-year period 1955–1958 inclusive it was found in five study plots in southeast Missouri that the incidence of oak wilt caused by the fungus, *Ceratocystus fagacearum* (Bretz) in black and scarlet oaks was significantly higher in trees that were blazed with an ax or wounded by boring auger holes in their trunks than it was in similar trees that were given a puncture-type wound or were not wounded. However, no infections developed in trees that were wounded before April 11 or after May 6. In all cases oak wilt developed the same season that the tree was wounded.

It seems likely that such insects as sapbeetles, wood roaches, and carpenter ants carried the inoculum to the trees that developed oak wilt. This is concluded because one or more of these insects was caught in traps that were stapled in auger holes on trees that developed oak wilt. However, the source of the inoculum is unknown, because no oak wilt fungus mats or other recognized sources of the fungus were found in systematic surveys made within and around the five plots.

Some researchers believe that insects have a major role in the overland spread of oak wilt. Oak wilt is a vascular disease that is caused by the fungus *Ceratocystus fagacearum* (Bretz) Hunt which Bretz (1955) found to be pathogenic on all species of native and exotic oaks that were inoculated with the fungus. Bart (1957) found that *C. fagacearum* is also pathogenic and produces symptoms similar to oak wilt in jonathan and delicious apples, and has survived without producing symptoms in ash, beech, dogwood, hophornbeam, hickory, sassafras, sourwood and wild cherry. It appears that in order for oak wilt to develop, the pathogen must have access to the vascular system of susceptible hosts. This may occur via wounds of various kinds. This disease of oaks is distributed over all or parts of 18 states (fig. 1), but is not known to occur outside the boundaries of the United States.

In trees of the red oak group, oak wilt symptoms are usually characterized by a sudden discoloration in the foliage. As a rule, the topmost leaves are the first to turn yellow and within a few days all of the leaves may be discolored. Leaf discoloration progresses from the margins toward the midrib and there is a tendency for the leaves to curl or cup. The leaves fall soon after they turn yellow. In trees of the white oak group, oak wilt symptoms are less dramatic in that foliage discoloration is usually more or less localized in one or more branches, and some trees apparently overcome the disease.

Evidence that wounds may serve as infection courts in nature was found in Pennsylvania (Guyton 1952, Craighead *et al.* 1953), Iowa (Norris 1955), and Wisconsin (Kuntz & Drake 1957) where the incidence of oak wilt was significantly higher in wounded trees than in similar trees not wounded. In those areas the oak wilt fungus forms what have been called "mats" (fig. 2a) under the bark of many trees that develop oak wilt. The bark above the larger mats often cracks (fig. 2b), and insects such as sapbeetles and vinegar flies gain access to the mats via the cracks in the bark. The mats emit a fruity odor that attracts such insects, and since these same insects are also attracted to fresh wounds it is logical to assume that oak wilt may be spread in this manner.

Tree Wounding and Insect Trapping Studies.—In Missouri the source of inoculum is not so obvious because oak wilt fungus mats are rare, and it is unlikely that they are important sources of inoculum for the overland spread of the disease. However, oak wilt developed each year in a number of isolated trees indicating overland spread. So it was decided that studies should be made to find out (1) if the incidence of oak wilt was higher in trees that were wounded than it was in similar trees that were not, and (2) if specific insects visited wounds on trees that developed oak wilt.

The studies reported here were made in second-growth oak-hickory stands on the Sinkin Experimental Forest in Dent County, Missouri. The area is more or less typical of thousands of acres in the Missouri Ozarks. Oak wilt occurs in the general area, but the annual loss from it is very low. No oak wilt was known to have occurred within or adjacent to the specific five plots selected for these studies, and no evidence of oak wilt fungus mats or diseased trees could be found within or surrounding the plots.

Five 3-acre plots were established, each contining approximately 1,800 oak stems ½ inch and larger in diameter. In each of the five plots 360 black and scarlet oaks 4 to 12 inches in diameter at breast height were divided at random into 36 similar groups of 10 trees. Each group was assigned at random to 1 of 36 combinations of three types of wounds and 12 treatment dates.

The three types of treatments were: blazes, punctures, and no wounds or checks.

The blazes were made with a downstroke of a cruising ax. This made a wound about 2 inches on a side, and cut through two or more of the outer growth rings, but did not remove the chip. Four such blazes were made about equidistant around the stem 1 to 2 feet above the ground, and four more were made 3 to 4 feet above the ground. The upper blazes were offset so they were not directly above the lower ones.

The puncture wounds were made with a downstroke of a pointed miners pick. The pick cut through two or more of the outer growth rings. Eight such wounds were made comparable in location to the blazes described above.

The check trees were not wounded.

The first wounds were made March 29, 1955, and were repeated on other trees at weekly intervals through June 14, 1955. On each wounding date 10 trees in each of five plots were blazed, 10 were wounded with a pointed miners

[1] Based on material presented at the national meeting of the Entomological Society of America, Salt Lake City, Utah, 1958. Accepted for publication January 29, 1960.

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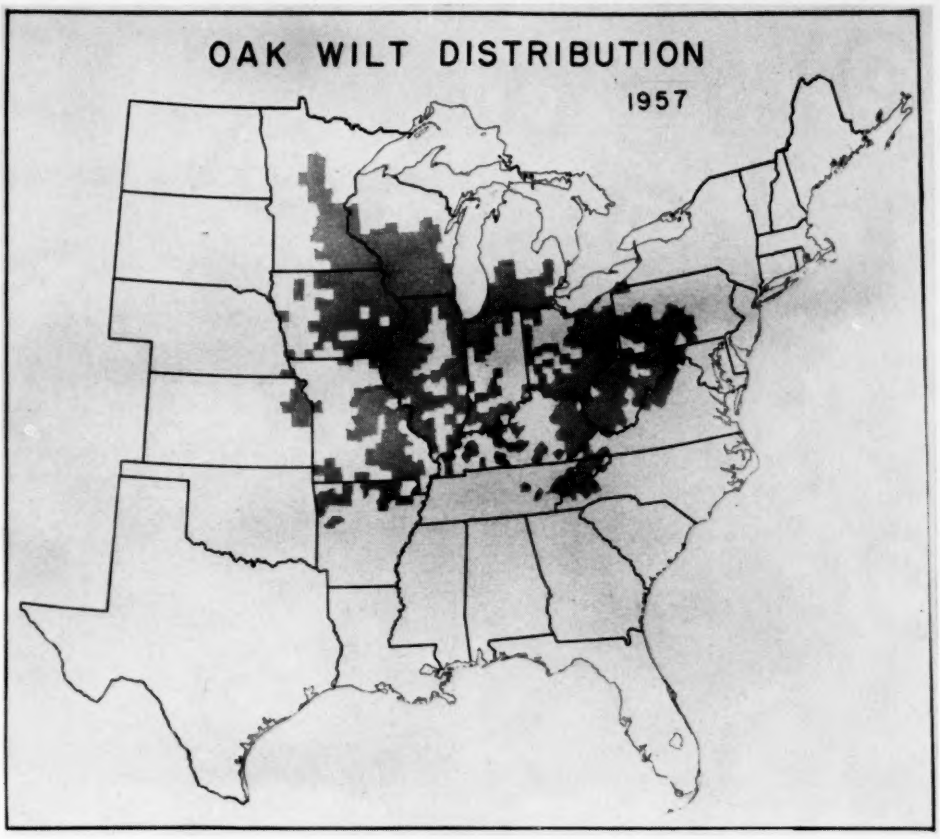


FIG. 1.—Distribution of oak wilt as of 1957.

pick, and 10 others were designated as nonwounded checks.

All trees within each plot were examined for oak wilt symptoms weekly through May and June, biweekly through September 15, 1955, and periodically during 1956 to 1958. Seven of the blazed trees developed oak wilt in 1955, and *C. fagacearum* was isolated from them.[3] Six of the diseased trees were blazed April 26 and one on April 19. Three of them were in one plot, and one was in each of the other four plots. None of the puncture-wounded or the check trees developed oak wilt. No trees in this study developed oak wilt after 1955.

In an analysis of variance it was found that the date and type of wound were highly significant variables affecting the incidence of oak wilt; *i.e.*, oak wilt developed only in trees that were blazed April 19 and 26.[4] Throughout the study large numbers of sapbeetles, vinegar flies, ants, cockroaches, et cetera, were observed on the blaze and puncture wounds.

To find out more specifically what species of insects visited wounds on trees that developed oak wilt, a specially designed trap was used in other wounding studies in 1956, 1957, and 1958 (fig. 3). This trap consisted of a screen cone inserted in an auger hole $1\frac{3}{4}$ inches in diameter bored $2\frac{1}{2}$ to 3 inches into the stems of the test trees. Immediately after a hole was bored, the small end of a cone was placed inside the auger hole and the large end was stapled to the smoothed bark surrounding the hole. Insects could enter the auger hole via an opening in the small end of the cone but had difficulty escaping.

An exploratory test of this trap was made in 1956. One hole was bored in each of the stems of 28 white, black, and scarlet oaks, and a cone, made from 32-mesh plastic screen, was stapled in each hole. Twenty-eight similar trees were nonwounded checks. Unfortunately some insects chewed holes in the plastic and may have escaped.

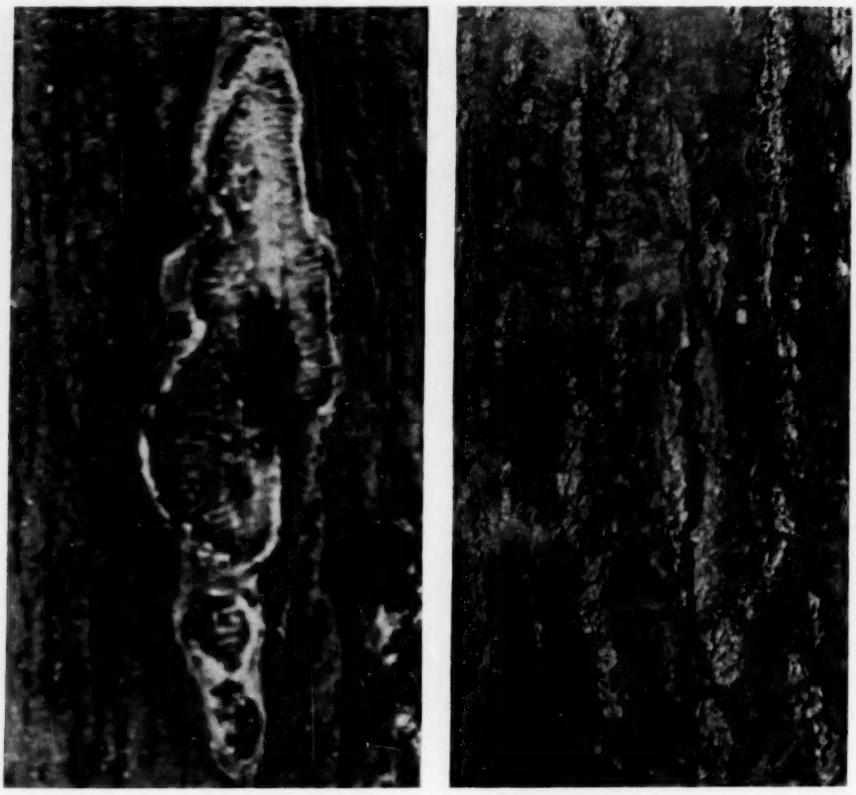
Oak wilt developed in one of the 28 auger-wounded trees but in none of the check trees. Cockroach nymphs and oak timberworm adults were found in the auger hole on the tree that developed oak wilt. Other insects may have entered and escaped via holes chewed in the plastic screen cone. Again it was demonstrated that the incidence of oak wilt was significantly higher in trees that were wounded than it was in check trees, but there is no assurance that the insects found in the holes were the only ones that entered.

An improved cone made from 60-mesh copper strainer cloth was used in the 1957 and 1958 studies. In trial tests with this trap no insects were observed to escape after they crawled through the hole in the small end of the cone.

On April 9, 1957, one auger hole was bored, and a cone was inserted in the stems of each of 10 trees selected at random from the check trees in each of four plots used

[3] All cultures were done by Dr. T. W. Bretz, Professor, School of Forestry, University of Missouri.

[4] All data analyses reported in this paper were made by Cecil L. Gregory, Associate Professor, Rural Sociology, University of Missouri.



a b

FIG. 2.—a. Oak wilt fungus mat after bark was removed.
b. Crack in bark caused by pressure of oak wilt fungus mat under it.

in the 1955 study. This was repeated at weekly intervals in other trees through May 7. An equal number of trees also selected at random was designated as checks. One, two, and three weeks after each hole was bored the cone trap was removed long enough to collect the insects found inside and record the number and groups to which they belonged.

Oak wilt developed in one of the 200 wounded trees but in none of the check trees. The tree that developed oak wilt was a 5-inch black oak that was wounded on April 16. At the end of the first week after the trap was installed two cockroach nymphs were found; at the end of the second week, one sapbeetle; and at the end of the third week, four cockroach nymphs and one darkling beetle were found.

The 1958 study was similar to the one in 1957 except that two auger holes were bored in each of 250 trees. These trees also were selected at random from black and scarlet oaks 4 to 12 inches d.b.h. in each of five plots. These trees had been check trees in the 1955 study. Twenty trees were wounded on April 8; 50 trees each on April 15, 22, 29, and May 6; and 30 on May 13.

Oak wilt developed in two of the wounded trees and in none of the check trees. One of the trees that developed oak wilt was a 9-inch d.b.h. scarlet oak wounded April 29. At the end of the first and second weeks after the holes were bored carpenter ants were the only insects found. At the end of the third week, one ant, six sapbeetles, one weevil, and one cockroach nymph were found.

The other tree that developed oak wilt was a 12-inch d.b.h. black oak that was wounded May 6. At the end of the first week 12 sapbeetles were found in the holes; at the end of the second week, three sapbeetles, one cockroach nymph, and one rovebeetle were found; and at the end of the third week, one sapbeetle, one cockroach nymph and one rovebeetle were found.

DISCUSSION AND CONCLUSIONS.—The results from these studies show that in an area where oak wilt fungus mats were rare or absent the incidence of oak wilt was significantly greater in trees that were blazed or wounded with an auger from April 11 to May 6 than in non-wounded trees. The disease developed in 7 of 600 trees that were blazed, 4 of 478 trees that were wounded with an auger, 0 of 600 that were wounded with a pick, and in 0 of about 15,000 check and other non-wounded oaks within and adjacent to the study area.

Five of the diseased trees were in one plot, two were in another, and one was in each of four other plots. There were no recognizable differences within the plots to account for the development of oak wilt in a significantly larger number of trees in this one plot. However, the following factors outside of this plot were different and may have a bearing. In 1953 the oak, hickory, and other hardwoods adjacent to one side of the plot were girdled and treated with herbicides. By 1955 most of the girdled

a b

FIG. 3.—a. Auger hole bored in tree.
b. Cone trap installed in auger hole.

trees were dead, and by 1958 all of them were dead and many had fallen. None of the trees had oak wilt symptoms when they were girdled, but from 1955–1958 a number of sapbeetles, cockroaches, ants, et cetera, were observed on some of the girdles that were moistened by fermenting sap and rain. Perhaps insects carried oak wilt fungus to the girdles and from there to wounds on trees within the plot.

It is assumed, until better evidence is produced, that insects carried the pathogen *C. fagacearum* to the trees that developed oak wilt. In the 1955 and 1956 studies the insects that visited the wounds on the test trees were able to come and go at will so that no particular insects could be associated with trees that developed oak wilt. But in 1957 it seems likely that the inoculum was carried by cockroach nymphs, and in 1958 carpenter ants are the likely carriers of the inoculum to one tree and sapbeetles to the other. If cockroach nymphs and carpenter ants did carry the inoculum it would have been necessary for them to obtain it in the immediate vicinity of the wounded trees because these insects cannot fly. The writer has no idea where any of the insects might have obtained the inoculum because no recognized source of inoculum was found.

These studies in Missouri suggest that only certain kinds of wounds made during a rather limited period of time may serve as infection courts in the overland spread of oak wilt. Perhaps a critical combination of factors is necessary for wounds to serve as infection courts in nature. If this is so it would account for the relatively limited spread of oak wilt.

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Increase of European Corn Borers Following Soil Application of Large Amounts of Dieldrin[1]

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ABSTRACT

During a 5-year period from 1954 through 1958, approximately 17,844 acres of Illinois farm land near Sheldon were treated with 2 and 3 pounds of dieldrin per acre for suppression of a local infestation of Japanese beetles (*Popillia japonica* Newm.). Surveys conducted each year in field corn showed that more European corn borers (*Pyrausta nubilalis* (Hbn.)) were present in fields within the treated area than in fields surrounding it. During 1954 and 1955, when corn borer populations were very high, fields within the suppression area averaged 2.6 more borers per infested stalk of corn than fields outside of the treated area.

During 1955, field corn growing within the treated area was slightly more attractive to the corn borer for egg deposition and for survival of borer larvae. Although more egg masses were laid and more corn borers survived in fields in the treated area, the slightly increased attractiveness of the corn in treated fields does not completely account for the great difference in number of European corn borers found in treated and untreated fields.

Some predators commonly found in cornfields were not adversely affected by the dieldrin. The corn borer parasite, *Lydella grisescens* R.-D., as well as some other common predators were equally abundant in the treated and untreated study areas.

The factors responsible for the increase in number of European corn borers in the large, dieldrin-treated, Japanese beetle suppression area at Sheldon, Illinois, were not definitely ascertained. The data presented tend to indicate that the size of the treated area was important and in some way contributed to the increase in number of corn borers which increase was not great enough to warrant additional control measures.

The widespread use of many new insecticides has often created new problems. Insect problems that develop following treatment with DDT, for example, have been generally recognized. Stevenson *et al.* (1944), Ebeling (1945), Wheeler & LaPlante (1946), and Sylvester (1949) are but a few of the researchers reporting an increase in aphids following the use of DDT. Increases in numbers of mites were reported by Ebeling (1945), Driggers (1946), and Wheeler & LaPlante (1946). Wilson (1949) and Wilson & Davis (1952) reported increased numbers of potato leafhoppers and pea aphids following treatment of alfalfa with chlordane, aldrin, and dieldrin.

Beginning in 1954 and continuing through 1958, dieldrin was applied by the Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture, and the State of Illinois Department of Agriculture for suppression of a local infestation of Japanese beetles (*Popillia japonica* Newm.) at Sheldon, Illinois. During these 5 years, greater than normal numbers of European corn borers (*Pyrausta nubilalis* (Hbn.)) were observed in the large area of farm land treated with dieldrin for suppression of the beetle. Dieldrin is very effective for "grub-proofing" the soil in the control, suppression, or eradication of the Japanese beetle, but little is known regarding the chemical's effect on other insects within the area that is treated. Thus, at the request of the cooperative agencies entomologists of the Illinois Natural History Survey obtained records on the abundance of some insects, including the European corn borer, within the control area and in the surrounding area at Sheldon.

PROCEDURES.—Beginning in 1954 and continuing through 1958, Federal and State agencies treated a total of 17,844 acres of Illinois farm land near Sheldon with 2 and 3 pounds of dieldrin per acre for suppression of a local infestation of Japanese beetles. In 1954, the chemical was applied to 1,500 acres by airplanes using sprays containing 3 pounds of dieldrin in 3 gallons of solvent or 10% granulated dieldrin broadcast at 30 pounds of granules per acre. Only granules were used in 1955 when 2,860 acres adjacent to and partly surrounding the 1954 block were treated with dieldrin. In 1956, 1957, and 1958, 13,484 acres were treated with dieldrin using 20 pounds of 10% dieldrin clay granules per acre. In addition, roadsides throughout much of the infested area were sprayed with 1 pound of DDT per acre during midsummer for control of adult Japanese beetles.

The dieldrin was applied during April and May each year. In plowed fields the chemical was often worked into the soil shortly after application, whereas in hay fields and permanent pastures the soil was not disturbed until the following year or it was not disturbed at all. Farmers in the area generally follow a 5-year rotation of corn, soybeans, small grains-legumes, and legumes.

During the 5-year period, observations were made on the number of European corn borers found in field corn in the treated and untreated area near Sheldon. The number of borers was estimated by examining 25 consecutive corn plants at two or four locations in each study field and recording the number of plants that appeared to be infested with corn borers. The first two plants found to be infested and the last two infested plants in each group of 25 were dissected for borers. Based on the results of the inspection and the dissection, the number of borers was calculated per 100 stalks of corn. The number of egg masses was determined by a complete examination of 25 plants in each study field. During 1955, a record of the growth and development of comparable fields of corn in the study areas was obtained using the tassel-ratio technique described by Luckmann & Decker (1952).

DISCUSSION OF DATA.—In October, 1954, the first year of treatment, two comparable fields or study areas were selected at 12 locations within and along the edge of the treated block. The results of this survey are presented in table 1. These counts show that in 1954, there were almost twice as many corn borers in fields in the treated area as there were in adjacent untreated fields.

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Table 1.—A comparison of the number of overwintering European corn borers found on October 18, 1954, in paired cornfields at 12 locations within and along the outside edge of the Japanese beetle control area at Sheldon, Illinois.

Outside the Area			Inside the Area[a]		
Untreated Field No.	Plants Infested with European Corn Borer (%)	Borers per 100 Plants	Treated Field No.	Plants Infested with European Corn Borer (%)	Borers per 100 Plants
1U	100	380	1T	100	660
2U	100	380	2T	100	660
3U	72	60	3T	100	1,120
4U	96	220	4T	96	680
5U	88	220	5T	96	280
6U	100	620	6T	100	620
7U	88	160	7T	100	460
8U	88	140	8T	100	660
9U	100	380	9T	100	1,100
10U	100	760	10T	100	700
11U	52	80	11T	100	440
12U	100	960	12T	100	500
Average	90	363	Average	99	657

[a] Soil treated with 3 pounds of dieldrin per acre.

These data are particularly interesting because fields 7T and 7U, table 1, are actually treated and untreated portions of the same field; yet 7T, the treated portion, contained 2.9 times as many corn borers as did the untreated portion. Likewise, field 8T and field 8U, table 1, are actually different portions of the same field. The 8T portion of the field was within the confines of the block treated with dieldrin in 1954, and it contained 4.7 times more borers than the untreated portion of the field.

The number of overwintering corn borers was determined each year during the 5-year period. Data from these surveys, table 2, show that the treated area always contained more borers than the surrounding untreated area. During the 5-year period, the difference in corn borers per infested stalk ranged from 2.6 borers per stalk in 1954 and 1955 to 0.5 borer per infested stalk in 1958.

The Japanese beetle infestation at Sheldon is located in Iroquois County, Illinois. A survey of the number of second generation corn borers per 100 stalks of corn is made annually within the county. These survey records, table 2, show that in 1954 the average number of corn borers in the combined treated and untreated study areas was the same as the population average for Iroquois County. In 1955, the combined treated and local untreated areas averaged fewer borers than found in the county-wide survey. In 1956, 1957, and 1958 the study areas at Sheldon averaged more corn borers. However, the county average was always below the average number of borers found in the dieldrin-treated area. While the difference in number of borers was not great, there was a consistent difference.

In 1955, counts were made of the number of first and second generation corn borer egg masses, the number of corn borer larvae produced per egg mass, and the growth and development of field corn in the treated and untreated areas. The data on egg counts and number of borers produced per egg mass, table 3, show that more egg masses were laid and more larvae survived per egg mass in the fields with the soil treatment of dieldrin.

Data on growth and development of 36 cornfields in June and July, 1955, table 4, show that the 12 study fields in the area treated in 1954 were slightly more advanced during the whorl, tassel, and silk stages. The fields in the area treated in 1955 appeared to be the slowest growing of the study fields although in these fields tasseling was slightly ahead of tasseling in the untreated fields in the study group. Thus, there was a slight difference in rate of development and attractiveness of the three groups of corn. The slight increase in attractiveness of the corn growing in the treated areas may account for an increase

Table 2.—A comparison of the number of overwintering European corn borer larvae found during September or October, 1954 through 1958, within and along the outside edge of the Japanese beetle control area at Sheldon, Illinois.

Year of Survey	Type of Area	Dosage of Dieldrin (Lbs. per Acre)	Stalks Infested with 2nd Generation European Corn Borer (%)	Average Number of Corn Borers Per Infested Stalk	Difference, Borers per Infested Stalk	No. of 2nd Generation European Corn Borers per 100 Stalks	Average Number of 2nd Generation European Corn Borers per 100 Stalks in Iroquois County,[c] Illinois
1954	Treated[a]	3	99	6.6		657	511
1954	Untreated[b]	0	90	4.0	2.6	363	
1955	Treated	3	99	8.6		857	839
1955	Untreated	0	98	6.0	2.6	586	
1956	Treated	2	88	2.5		224	88
1956	Untreated	0	71	0.8	1.7	82	
1957	Treated	2	72	1.4		104	44
1957	Untreated	0	72	.8	.6	58	
1958	Treated	2	69	1.4		100	48
1958	Untreated	0	86	.9	.5	75	

[a] Control area.
[b] Surrounding area.
[c] The Japanese beetle control area at Sheldon, Illinois, is located in Iroquois County.

Table 3.—Numbers of first and second generation European corn borer egg masses and larvae found during 1955 in 24 fields of corn within and along the periphery of the Japanese beetle control area at Sheldon, Illinois.

Borer Stages	First Generation[a]			Second Generation[a]		
	Area treated in		Adjacent Outside Area	Area treated in		Adjacent Outside Area
	1954	1955		1954	1955	
Egg masses	4.85	2.86	3.16	3.77	2.90	2.48
Borers per plant	2.67	2.03	1.11	8.08	9.06	5.86
Larvae per egg mass	.64	.76	.34	2.14	3.12	2.36

[a] Based on examination of eight fields in each area.

in corn borers, but the increase in attractiveness does not seem of sufficient magnitude to account completely for the great difference in number of corn borers found in treated and untreated fields.

Farmers in the suppression area at Sheldon often implied that corn yields were excellent despite the greater abundance of corn borers. Normal treating for the corn borer was done during 1954 and 1955, but the soil treatment with dieldrin did not create or aggravate any economic problem and no additional or supplemental treating was done for any insect pest. In fact, many insects that come in occasional or frequent contact with the soil were quickly eliminated, and common insect problems of field and forage crops did not occur on the farms that were treated with dieldrin.

The dieldrin appeared to have little adverse effect on some of the common predators of corn borers and corn borer egg masses. Adult and immature Coccinellids were quite abundant, and Nabids, Chrysopids, and Syrphids appeared not to be adversely affected by the treatment of dieldrin. The egg predator, *Orius insidiosus* (Say), appeared to be equally abundant in the treated and in the surrounding untreated areas. The extent of parasitism by *Lydella grisescens* R.-D. was not affected by the dieldrin.

In untreated fields adjacent to the Japanese beetle control area, parasitism of corn borers by *Lydella grisescens* in 1954, 1955, and 1958 averaged 8.2%, 14.5% and 20%, respectively. Parasitism of corn borers in treated fields averaged 5.5% in 1954, 19.3% in 1955, and 16.7% in 1958.

Table 4.—Growth and development of field corn expressed as tassel ratio in two areas treated with dieldrin and one untreated area within and along the edge of the Japanese beetle control area at Sheldon, Illinois.

Date (1955)	Development of Field Corn Based on Tassel Ratio		
	Control Area Treated In		Adjacent Untreated Area Surrounding 1954 and 1955 Areas
	1954	1955	
June 10	5.6	4.9	4.9
June 15	6.0	5.5	5.5
June 21	8.4	6.0	7.3
June 27	20.5	15.0	17.2
July 2	40.0	34.9	36.7
July 7	60.2	51.2	56.4
	31% T-1[a]	23% T-1[a]	9% T-1[a]
July 12	74.0	71.0	72.5
July 21	98.9	95.0	94.0

[a] Plants classified as T-1 are just beginning to tassel.

Table 5.—A comparison of the number of European corn borers and yield of ear corn in two special treatment areas within 4 miles of the Japanese beetle suppression area at Sheldon, Illinois, in 1955.

Plot Identification	No. of Borers per 100 Stalks		Average Yield of Ear Corn in 50′ of Row		
	1st Generation	2nd Generation	No. of Ears	Field Weight (Lb.)	No. 2 Corn (Lb.)
Untreated	82	394	44.7	17.3	20.4
Dieldrin, 2 lb.[a]	78	452	45.7	21.2	22.3
Untreated	85	470	40.0	19.8	19.8
Dieldrin, 2 lb.[a]	85	420	46.0	25.7	20.4

[a] 20 lb. of 10% dieldrin clay granules per acre.

Parts of two fields of corn outside the suppression area at Sheldon were treated with dieldrin to observe whether differences in number of corn borers always occurred with a soil application of dieldrin. Both test fields were within 4 miles of the large block treated with dieldrin. The results of these tests, table 5, show that there was no difference in the number of borers when the treatment was applied on an individual field basis with the surrounding fields left untreated. There was a difference in yield of corn in favor of the dieldrin-treated portion of each field. Thus, these data tend to indicate that the size of the treated area was important and in some way contributed to the increase in numbers of European corn borers.

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Influence of Soil and Moisture on the Beet Webworm, *Loxostege sticticalis*, and Its Parasites[1]

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Abstract

The effect on survival and emergence of the beet webworm, *Loxostege sticticalis* (L.), of surface exposure and burial to 2 and 6 inches in 3 soil types at various moisture levels was measured in the laboratory.

Mortality of webworms in the prepupal and pupal stages increased as the burial depth and moisture content increased. Depth of burial had the greatest influence on adult emergence; only three moths emerged from buried cocoons whereas many parasites emerged from the 2- and 6-inch depths. Consequently, tillage to bury the cocoons would help prevent webworm emergence and, at the same time, preserve many of the parasites.

Outbreaks of the beet webworm, *Loxostege sticticalis* (L.), lasting for several years have occurred periodically in the prairie region of Canada. In years of high larval density the incidence of parasitism also may be high. In Montana, Pepper & Hastings (1941) reported that one sample of 200 larvae was 98% parasitized. During an outbreak of the beet webworm, cultural practices may be of value in controlling it as well as in preserving its parasites. A laboratory study was undertaken to measure the effects on survival and emergence of both the webworm and its parasites of burial of webworms in cocoons at various depths in different soil types and under a range of soil moisture levels.

Methods and Materials.—Webworm cocoons collected at Picture Butte, Alberta, in the fall of 1949 were exposed at the surface or covered with 2 or 6 inches of sand, silt-loam, or clay in pint and quart glass jars during the winter of 1949–50. The total depth of soil was at least 3 inches to provide an adequate volume to minimize changes in the moisture content. Soils that had been stored in a dry atmosphere for 2 months were used. Moisture was determined by weight; where needed water was added to the desired soil quantity with an atomizer to obtain the following percentages: 0, 5, 9, 13, and 17 in the sand; 3, 5, 9, 13, 17, and 23 in the silt-loam; and 4, 5, 9, 17, 23, and 29 in the clay. The lowest percentage in each soil type was that of the air-dried soil, and the highest was within 1% of the saturation point of that type as judged by the occurrence of free water on the surface after standing for several hours. Each jar was closed with a glass lid and weighed. Twelve days after the first weighing the jars were weighed again and water was added to those that had lost weight. About 10% of the jars required 5 to 10 ml. of water.

A single jar contained 23 cocoons in one position, in one soil type, and at one moisture level. Combinations of moisture level and position of cocoons were set up for a single soil type at one time. This was repeated three times for each soil type, so that 1,035 cocoons were used in the sand and 1,242 each in the silt-loam and clay.

Emergence was recorded daily. After 28 days the soil was examined for unemerged insects and cocoons were dissected.

Results.—*Webworm.*—Percentage mortality of the webworms in the prepupal and pupal stages was:—

Position	*Sand*	*Silt-loam*	*Clay*
Surface	33	21	23
2 inches deep	43	43	32
6 inches deep	48	49	40

The increased mortality at greater depths could have been the effect of greater compaction of the soil. However, the greatest effect on mortality of the immature webworms was that of moisture content. The percentages dead in the three soil types at different moisture levels were:—

Percentage Moisture	*Sand*	*Silt-loam*	*Clay*
Air-dried	21	22	35
5	30	24	26
9	35	29	28
13	52	38	—
17	67	42	26
23	—	71	32
29	—	—	44

Mortality increased progressively with increased moisture in the sand and silt-loam but remained nearly uniform in the clay except for the lowest and highest levels of moisture. The increase in mortality with increase in moisture was probably the result of favorable conditions for micro-organisms, as many of the prepupae had almost totally disintegrated during the 28 days of exposure.

Still alive at the end of the tests were 12%, 7%, and 8% of the prepupae in sand, silt-loam, and clay, respectively.

Some adult webworms were found dead both in the cocoons and in the soil. The figure amounted to about 30% in cocoons on the surface and almost 100% in cocoons buried at 2 and 6 inches (fig. 1).

Parasites.—Parasitism in cocoons used in the test was slightly over 31%. The percentages of species of 686 parasites that reached the adult stage were:

Meteorus loxostege Vier.	41
Pristomerus appalachianus Vier.	18
Cremnops vulgaris (Cress.)	17
Mesochorus perniciosis Vier.	15
Campoplex sp.	9

An additional 415 immature specimens were not identified; of these, 226 were still alive at the end of the tests. *Mesochorus perniciosis* Vier. was a hyperparasite probably of at least two of the above species of primary parasites and possibly of all four (Simmonds 1947a, 1947b).

Soil type had little effect on parasite survival but moisture had considerable effect. Percentage mortality of the parasites in immature stages varied in the sand,

[1] Contribution from the Entomology Section, Canada Agriculture Research Station, Lethbridge, Alberta. Accepted for publication February 2, 1960.

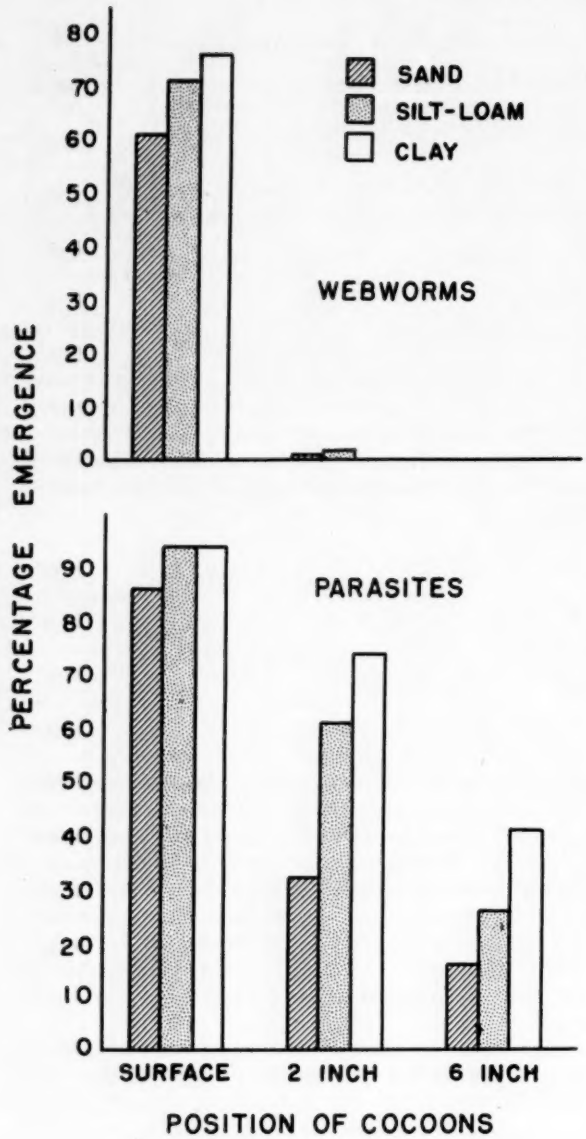

FIG. 1.—Emergence from various soil depths of beet webworms and parasites that had reached the adult stage.

silt-loam, and clay from 1, 2, and 0 in the dry soils to 11, 5, and 11 in the near-saturated soils. The greatest mortality in the silt-loam was 11% also, but it occurred at the 17% moisture level.

Percentage of emergence of those parasites that reached the adult stage decreased with depth of burial as did that of the webworm (fig. 1) but not to such a marked extent. Soil type had some influence; possibly the structure of the clay and loam soils prevented these soils from collapsing against the insects as they attempted to reach the surface.

DISCUSSION.—Results indicate that burial of beet webworm cocoons to a depth of 2 to 6 inches will nearly prevent webworm emergence but will still permit considerable parasite emergence. Hence, it would seem advisable from these results to apply a type of tillage that would bury the cocoons to a depth of at least 2 inches. This would prevent webworm emergence but still ensure adequate parasite emergence to be effective the following year. Other measures such as irrigation to control moisture level or cropping on particular soil types would not have sufficient influence on emergence of webworms to be of general value.

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Commerical and Experimental Organic Insecticides by Eugene E. Kenaga. This worthwhile paper appeared in the June, 1960 issue of the BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA. Reprints with covers are available, postpaid, at the following prices:

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The Biology of the Pine Webworm, *Tetralopha robustella*, in Wisconsin[1]

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ABSTRACT

The pine webworm, *Tetralopha robustella* Zeller, is a potential pest in Wisconsin as a defoliator of red and jack pines. Its life history and habits were studied in a plantation of red pine and a natural stand of jack pine on the sandy Wisconsin River valley. The webworm is widely distributed throughout the eastern half of the United States, and has been recorded on 10 species of pine.

An analysis of head capsule measurements using Dyar's Rule indicates six instars. A general description of eggs, larvae, cocoons, and adults of the webworm is given. Webworm larvae overwinter in sand-covered cocoons within the first inch of soil beneath the host tree. Pupation occurs in early June and adults emerge about two weeks later. Oviposition begins in early July and continues until mid-August. Larvae appear in early July and spend at least the first instar mining needles. Then they leave the needles and construct frass shelters in which several live for the remainder of the larval feeding period. They begin to abandon the nest to enter the ground to overwinter in mid-September; some remain in the frass nest until mid-November.

Eight parasites and two insect predators were found feeding on the webworm. Twig and branch foraging birds were also observed destroying frass nests and eating webworms.

The pine webworm, *Tetralopha robustella* Zeller, (Lepidoptera: Pyralidae) is a potentially serious defoliator of red and jack pine in Wisconsin plantations. Large populations of the webworm have built up periodically and severe defoliation and stunting of pines has been reported throughout the Lake States. Often, needle-feeding has been of less immediate concern than the ugly frass nests (fig. 1) formed on the twigs and small branches of its host trees. Owners of young red pine plantations have become increasingly concerned over the presence of these frass masses as their trees approach favorable Christmas tree size. The investigations reported here were undertaken to provide information on the biology of the webworm in Wisconsin.

SYSTEMATIC HISTORY.—*Tetralopha robustella* was described with other species of the same genus by Zeller (1848) from specimens collected in Texas. He regarded species of *Tetralopha* as Phycitids. Grote (1878) published a systematic synopsis of the species described by Zeller and gave the name Epipaschiae to the group. He characterized this group by the flattened clypeus and the scaled process attached to the base of the antenna in the male, which extends backward over the thorax. Currently, the Epipaschiae (now Epipaschiinae) is a small subfamily, transitional between the Pyralidinae and the Phycitinae in the family Pyralidae Forbes (1925).

Grote (1879) described a new species, *T. diluculella*, from specimens collected in New York. Comstock (1880) and Packard (1890) also used *T. diluculella* in discussions of the webworm. Britton (1919) stated that *T. diluculella* is a synonym of *Tetralopha robustella* Zeller. Two other species of *Tetralopha* have been recorded as pests or potential pests of forest or ornamental trees: *T. asperatella* Clemens has been found feeding on oak, maple, elm, and beech; *T. melanogrommis* Zeller has been reported attacking white pine (Herrick 1935).

DESCRIPTION OF THE STUDY AREA.—A study area was established on a 40-acre plantation of red pine growing on the outwash plain of the lower Wisconsin River. The terrain was level, providing continual exposure of the trees. The soil was sandy to a depth of at least five feet. Drainage was good and water did not stand long after a rain. The five-year-old seedlings were spaced 4×4 feet, and at the time of the study were about two feet high. They were in good vigor and apparently free of pests other than the pine webworm. The ground cover consisted of short prairie grasses except in two large sand blow areas where the trees were stunted, tipped over, and in some cases, had their roots exposed as a result of the sands being shifted frequently by strong winds. Adjoining the eastern edge of the plantation was a 40-acre tract of natural jack pine trees from 2-foot seedlings to 15-foot saplings. Practically all trees were infested with the webworm. These trees were not vigorous and were infested with the jack-pine budworm, *Choristoneura pinus* Freeman, and the pitch blister moth, *Petrova albicapitana* (Busck).

DISTRIBUTION AND HOSTS.—The pine webworm is distributed throughout the eastern half of the United States wherever pines are grown. The following pines have been reported infested by the webworm:[4]

Lake States:	
Pinus banksiana Lamb.	jack pine
P. resinosa Ait.	red pine
P. strobus L.	eastern white pine
P. sylvestris L.	Scotch pine
Northeastern States:	
P. rigida Mill.	pitch pine
P. resinosa Ait.	red pine
P. sylvestris L.	Scotch pine
P. virginiana Mill.	Virginia pine
Southern States:	
P. elliottii Engelm.	slash pine
P. echinata Mill.	shortleaf pine
P. palustris Mill.	longleaf pine
P. taeda L.	loblolly pine

In the Lake States jack pine is the preferred host, although red pine also may be severely defoliated. Eastern white pine is attacked lightly. In the Northeastern States pitch pine is the principal host and other pines are less frequently infested. All four hard pines grown in the Southern States are defoliated.

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FIG. 1.—Nest of the pine webworm on jack pine.

DESCRIPTION OF LIFE STAGES.—*Eggs.*—The eggs are ovoid, flattened, and about 1 mm. long. They are covered with a translucent, reticulated chorion that imparts a glossy sheen to their surfaces. Eggs are cream colored when freshly laid, but turn bright canary yellow prior to hatching. The chorion remains as an empty "shell" on the pine needles after the larvae have emerged.

Larvae.—Number of instars: An evaluation of larval head capsule width measurements, employing Dyar's Rule, suggests there are six larval instars. In this study, 680 measurements were extremely variable, but fell into groups around the six values 0.37, 0.53, 0.73, 0.97, 1.33, and 1.83 millimeters. A constant ratio was obtained when each of these values was divided by the succeeding one:

Instar	*Head capsule ratio*
2nd	.37/ .53= .70
3rd	.53/ .73= .73
4th	.73/ .97= .75
5th	.97/1.33= .73
6th	1.33/1.83= .73

When these head capsule data were subjected to a frequency distribution analysis, a seventh instar was revealed. Unfortunately, efforts to rear webworm larvae individually to obtain a series of cast skins were not successful and the true number of larval instars was not established.

First instar larvae are about 2.5 to 3.0 mm. long. Their ground color is grayish-yellow, whereas the head and prothoracic shield are very light brown. Ten reddish-brown stripes run the length of the body from the prothorax to the ninth abdominal segment.

The ultimate instar is 13 to 16 mm. long, light brown in ground color with darker subdorsal and supraspiracular stripes extending from the pronotum to the ninth abdominal segment. The head is light brown with near-black, conspicuous, pigmented areas on the dorsal and caudal portions (Peterson 1951).

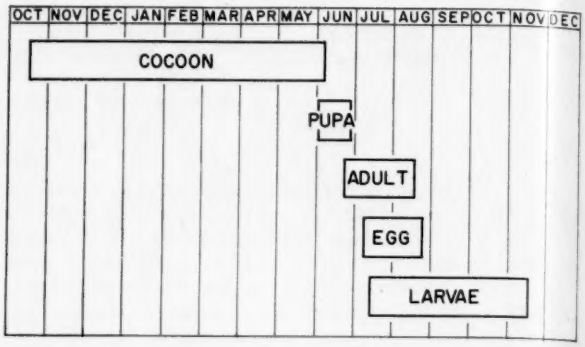

FIG. 2.—Seasonal cycle of the pine webworm in Wisconsin.

Cocoons are flattened, ovoid cells 12.3 by 8.0 mm. They are covered with a tightly packed lattice of sand grains, and lined with yellow silk.

The description of the adult is paraphrased from Forbes (1925) as follows:

"The adult is 22 mm. across its spread wings. The basal segment of the antenna is enlarged in both sexes. The male is without the scaled process characteristic of the subfamily Epipaschiinae. The basal third of the forewing is black, paler at the extreme base, and bordered distally by a fine, white line. The remainder of the forewing is pale gray, shading to darker gray toward the outer half. The hind wings are brown, with darker shading toward the outer margin, and a dark brown line at the base of the fringe on the outer margin."

SEASONAL HISTORY AND HABITS.—Pine webworm larvae construct their overwintering cells beneath the host tree, almost directly below the evacuated frass nest. Cocoons are spun within the first inch of soil, although depth seems to be influenced by compactness of the soil. In southern Wisconsin, overwintering larvae transform to pupae within the cocoon in late spring, usually during the first two weeks of June (fig. 2).

Webworm adults emerge from their overwintering cells about two weeks after pupation. Mating occurs, and the first eggs of the season may be found within the first two weeks of July. Eggs are laid most commonly in single rows along the flat surface of jack and red pine needles. Each egg in the row slightly overlaps the adjacent one toward the base of the needle, the entire cluster suggesting a row of raised fish scales. A regular pattern of oviposition is not adhered to by the female, and very often these egg rows appear as irregular egg masses. A single row or mass contains an average of 3.5 eggs with a range from 1 to 22 eggs. Occasionally, single eggs may be laid on needles, the twig bark of jack pine, and in some instances, on the needle bracts on jack pine twigs.

On jack pine, oviposition occurs most frequently on needles near the base of the previous year's shoot. This habit facilitates expansion of the larval colony toward the tip or base of the infested lateral branch. On red pine, eggs are concentrated on needles near the main stem at the point where lateral branches occur.

A number of egg rows or masses usually occur closely associated on adjacent needles of a jack pine twig. Each group of associated masses probably represents the eggs of

a single female, and the larval hatch from each group share a common frass nest. The number of eggs per group increased to 101.5 on July 19, after which there was a steady decline until August 15.

First instar larvae were observed July 10 and hatching continued to August 15. Small larvae wander about for a short time in the immediate vicinity of the eggs. They lay down a fine meshwork of silk strands among adjacent needles. Then they cut a circular hole, somewhat greater in diameter than the larval head, in the epidermis of the needle close to its base. A single larva enters, begins feeding, and works its way toward the tip of the needle. Frass pushed out through the entrance hole piles up in a continually growing mound of pellets that completely occludes the hole. It appears that larvae molt only once while mining needles. When webworms outgrow the confines of mined needles, they generally cut out through the needle epidermis. Mined needles, still intact, indicate that some larvae leave through the entrance hole.

After the larvae abandon the mined needles, they begin constructing the frass nest that will provide them with shelter during the remainder of their feeding period. Each larvae begins laying silk strands among adjacent needles on a twig, thus forming a skeletal framework that will later support the mass of frass pellets. The construction of a single nest, which will provide shelter for a considerable number of webworms, involves the labor of several individuals. An individual webworm delays formation of the nest and wanders about for some time, apparently in search of other webworms or a developing nest.

A completed frass nest contains a number of ramifying silken tubes within which larvae move about rapidly. A larva crawls only part way out of the nest to feed while maintaining its "footing" in one of the silk lined runways; it then clips a nearby needle at its base and pulls it into the nest to feed on it. When the needles close to the nest are consumed, larger larvae wander out along a twig to forage, often as far away as six inches from the nest. Their paths along the twigs are strewn with strands of silk, and in some cases, complete silken tubes run from the frass nest to the food source.

Feeding activity apparently is not limited to any particular time of day, although larvae were most active during periods when slight overcast eliminated direct sunlight on the nest, and when there was little or no wind moving the branches. Generally, any slight nearby movement disturbs a webworm and causes it to draw back into the nest.

The number of larvae per frass nest reached a peak of 78.0 on August 2 and by September 17 there were only 10.7 larvae per nest. This decline may be attributed to increased parasitism and predation or to emigration of larvae from the nest. This latter phenomenon seems common, and large larvae forage some distance from their original communal nests, and eventually establish their own frass shelters.

The first larvae to drop from trees began spinning cocoons on September 17. Some larvae remained on the trees until mid-November before entering the ground. One generation of the webworm occurs on the average in southern Wisconsin. A second generation of adults emerged from cocoons formed in the laboratory in September. No moths were observed in the field at this late date. It is doubtful whether second generation larvae would be able to complete their full cycle before a killing frost occurred, if indeed, the moths would actually mate and lay eggs.

NATURAL CONTROL.—Two hymenopterous parasites in the family Eulophidae were collected early in the season from larvae in mined needles: *Sympiesis sp.* was collected July 22; and *Derostenus silvia* (Grlt.) July 28. These species were found in the pupal stage within needles mined by webworms.

Many overwintering webworms were infested with larvae of *Apanteles* at the end of the season. Five adult parasites emerged from the same cocoon collection: two larvaevorids, *Phorocera* (*sens. lat.*) *tortricis* (og., and *Phorocera sp.*; three ichneumonids, *Trachysphyrus albitarsis albitarsis* (Cresson), *Mesostenus thoracicus* Cress., and *Zaleptoygus sp.*[5]

Throughout the larval feeding season *Acholla multispinosa* (DeG.) (Reduviidae) commonly was observed feeding on webworms and resting on frass nests. Spiders also were observed on the frass nests but none were ever seen feeding on larvae.

Birds, which forage in tree bark and among the branches and twigs of conifers, fed on webworms in the higher branches of jack pine. Chickadees and nuthatches were most commonly seen tearing the frass nests apart to obtain larvae.

In the fall, tiny unidentified red ants actively attacked larvae spinning cocoons on the ground. These ants also fed on larvae that had spun their cocoons, but apparently only larvae in imperfectly constructed cocoons were attacked.

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[5] The authors are indebted to specialists of the Insect Identification and Parasite Introduction Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland, for parasite determinations.

European Corn Borer Control in Peppers[1]

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ABSTRACT

In recent years heavy infestations of the European corn borer, *Pyrausta nubilalis* (Hbn.), in peppers grown in the "Delmarva" area have greatly reduced yields. Additional losses have occurred through the rejection of infested peppers by inspectors at the canneries. Field experiments were conducted in 1959 to test dosages, formulations, schedules, and a sticker, primarily using DDT to control this pest. An improved "Slosser"-type spray boom especially designed to spray peppers was used for 11 randomized treatments, replicated 4 times, in 3 fields.

Excellent control of the European corn borer was obtained with DDT and Sevin but not with toxaphene. There were no significant differences between weekly and 10-day spray schedules, or between wettable powder and emulsion formulations of DDT, or between DDT at 1 or 2 lb./acre rates, or between DDT or Sevin® (1-naphthyl methylcarbamate) with and without a sticker (Plyac) in a 7-day schedule. Excellent control was achieved by the DDT-plus-sticker treatments, applied on a 10-day schedule, suggesting that spray intervals might be lengthened beyond the weekly spray periods. Observations on timing of sprays and borer development in Delaware indicated the need for continuous insecticide protection for peppers during periods of adult flights, egg deposition and emergence of young larvae.

Heavy infestations of the tobacco hornworm, *Protoparce sexta* (Johan.), and the cabbage looper, *Trichoplusia ni* (Hbn.), were very common in the untreated plots. Practically no infestations of these two species occurred in the DDT and Sevin treatments. Green peach aphid, *Myzus persicae* (Sulz.), populations were very high during August and September, particularly in the DDT treatments. Malathion applied at the recommended rates failed to control them.

The economic losses from attacks of the European corn borer, *Pyrausta nubilalis* (Hbn.), on peppers have reached a point where its control is essential if peppers are to continue as a profitable crop in Delaware and in parts of Maryland and Virginia. Serious losses occurred in Delaware in 1956, 1958 and again in 1959. Inspection of many pepper fields during the '58 and '59 seasons indicated an average loss owing to borer infestations of from 40% to 50% of the entire crop. Many fields had 75% to 100% of the pepper fruits infested at the time of inspection. See figure 1 for typically infested pepper.

The seriousness of the problem prompted a cooperative study by research personnel of the Universities of Delaware and Maryland and the Virginia Truck Experiment Station. The first year's results of field experiments carried on in Delaware are the subject of this paper.

In recent years a serious problem developed with this pest in pimento peppers in Alabama. Based on three season's field work dusts of 20% toxaphene, or 2% endrin, or 10% DDT, applied at weekly intervals beginning about July 1 and at 4- or 5-day intervals during the month of August are now recommended (Arant 1958). Preliminary field experiments by MacCreary & Connell (1958) in Delaware using a row-crop sprayer equipped with three nozzles per row and DDT applied at the rate of 1 pound to the acre, indicated that corn borer infestations in peppers decreased as the number of sprays were increased. Treatments applied five times, at 2-week intervals, showed more than a two-fold increase in yield in comparison with the check area.

METHODS AND EQUIPMENT.—The experiments in Delaware were designed to test dosages, formulations, schedules, and a sticker, primarily using DDT in an attempt to control the European corn borer. Eleven randomized treatments, replicated four times, were laid out in three pepper fields (hereafter referred to as Fields A, B, and C) near Bridgeville, Delaware. The plots, 132 in all, were four rows wide and 50 feet long. A 20-foot roadway separated each replication. In Fields B and C each plot was separated from the adjoining plot by pulling up every fifth row of plants whereas in Field A each fifth row was left standing and untreated throughout the season. The pepper plants were California Wonder variety transplants of local origin.

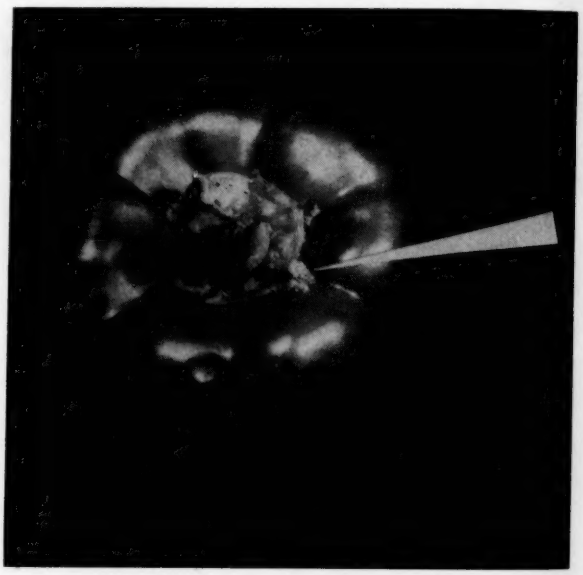

FIG. 1.—Pepper infested with European corn borer. Arrow points to borer frass.

Borer infestation data were taken from the two middle rows of each plot, to within 10 feet of each end. All red peppers were picked at each harvest, broken open and carefully examined for borers. The first six harvests from Fields A and B were red peppers and the seventh was a

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Table 1.—European corn borer control in California Wonder peppers, Bridgeville, Delaware, 1959.

TREATMENT	RATE/A. (LB. ACTUAL)	AVERAGE PER CENT OF PEPPERS INFESTED PER HARVEST							MEAN ANGLES[c]
		1	2	3	4	5	6	7	
				Field A					
Check	—	7.9	41.5	46.1	35.6	22.9	7.1	6.4	27.90
DDT, w.p.	1	0.0	1.0	3.9	0.0	0.0	0.0	1.3	2.79
DDT, w.p.	2	0.0	0.5	2.8	0.0	0.0	0.0	0.0	1.14
DDT, w.p.[a,b]	2	1.4	0.5	0.6	0.0	0.0	0.0	0.8	1.54
DDT, w.p.[a]	2	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.29
DDT, e.c.	1	0.0	0.5	2.4	0.0	0.0	0.0	0.0	0.93
DDT, e.c.	2	0.0	1.0	0.8	0.0	0.0	0.0	0.0	0.72
DDT, e.c.[a,b]	2	0.0	0.5	2.5	0.0	6.2	0.0	0.0	2.10
Sevin, w.p.[a]	2	0.0	0.0	0.6	1.2	2.1	0.0	0.0	1.50
Sevin, w.p.	2	1.3	0.0	1.2	1.0	0.0	0.0	0.0	1.41
Toxaphene, e.c.	2	1.4	10.0	8.3	19.0	6.1	0.7	0.0	9.64
L.S.D. at 5% level									2.73
at 1% level									3.59
				Field B					
Check	—	0.7	24.5	22.1	24.1	36.1	10.9	7.0	21.89
DDT, w.p.	1	1.4	1.5	1.1	0.0	0.0	0.0	0.0	1.43
DDT, w.p.	2	0.0	0.5	0.8	0.0	0.0	0.0	0.0	0.67
DDT, w.p.[a,b]	2	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.29
DDT, w.p.[a]	2	0.0	0.5	0.0	0.0	0.0	0.0	0.8	0.84
DDT, e.c.	1	0.0	1.0	2.3	0.0	2.9	0.6	0.0	2.11
DDT, e.c.	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
DDT, e.c.[a,b]	2	0.0	1.0	0.0	0.0	0.0	0.5	1.5	1.53
Sevin, w.p.[a]	2	0.5	1.0	0.0	6.6	0.0	0.2	0.0	2.35
Sevin, w.p.	2	0.9	0.0	0.0	0.0	2.0	0.0	0.0	2.12
Toxaphene, e.c.	2	0.0	8.5	11.7	14.3	10.6	1.0	0.5	9.25
L.S.D. at 5% level									3.08
at 1% level									4.06
				Field C					
Check	—	2.0	27.5	21.2					27.71
DDT, w.p.	1	0.0	1.0	0.0					1.62
DDT, w.p.	2	0.0	0.0	0.0					0.00
DDT, w.p.[a,b]	2	0.0	0.0	1.8					1.39
DDT, w.p.[a]	2	0.0	0.0	0.0					0.00
DDT, e.c.	1	0.0	0.7	2.5					2.69
DDT, e.c.	2	0.0	0.0	0.0					0.00
DDT, e.c.[a,b]	2	0.0	0.0	0.0					0.00
Sevin, w.p.[a]	2	0.0	0.9	0.0					1.71
Sevin, w.p.	2	0.0	0.0	0.0					0.00
Toxaphene, e.c.	2	5.1	2.0	8.7					7.01
L.S.D. at 5% level									5.98
at 1% level									7.98

[a] Plus Plyac—4 oz./A.
[b] 10-day spray schedule, the remaining treatments sprayed at weekly intervals.
[c] Percentages were transformed to angles in data analyses (G. W. Snedecor 1950).

green pepper, marketable size only, harvest. Field C, which had a very low yield, was only harvested three times. Yields during the second harvest for Fields A and B were so high that only 50 peppers were examined from each plot.

The insecticides were applied by an improved or "Slosser" type spray boom, especially designed and constructed to spray peppers by L. P. Ditman of the Entomology Department and G. J. Burkhardt of the Agricultural Engineering Department of the Maryland Agricultural Experiment Station. The spray boom, equipped with a hydraulic lift, was mounted on a Farmall H-type tractor. It was designed to spray four rows simultaneously, each row with nine nozzles. Six nozzles, three on each side of a row, were arranged close to the ground and directed upward. Two nozzles were in a lateral position and directed inward. A single nozzle was placed at the center and top of each row and directed downward. One potato-type nozzle with a number two disc was situated in the middle of each group of three nozzles at the ground level and the remainder were flat T-jet spray nozzles. The sprayer was operated at 250 p.s.i. pressure and delivered 9.38 gallons per minute. A 20-gallon-per-minute pump was used and the return from the pump to the 50-gallon spray tank produced sufficient turbulence to insure good agitation. Tractor speed was 3.5 miles per hour and application was at the rate of 100 gallons per acre. The spray thoroughly wet the plants both from above and below.

The initial application was on July 9 for Fields B and C and July 23 for Field A. Treatments 4 and 8 (the 10-day schedule) were sprayed seven times. The remaining treatments were sprayed on a weekly schedule with Field A receiving 9 applications, Field B 10, and Field C 8.

RESULTS OF FIELD TESTS.—A summary of the infestation data for all the treatments for each harvest in the

three fields is presented in table 1. Excellent control of the European corn borer was achieved by all the insecticide treatments except toxaphene. The results of an analysis of variance of the data is as follows: 1. All the insecticide treatments were significantly better at the 1% and 5% level than the untreated checks. 2. There was no significant difference between any of the DDT or Sevin treatments at the 1% and 5% level. 3. Toxaphene was significantly poorer than all the DDT and Sevin treatments at the 1% and 5% level in Fields A and B. In Field C, probably owing to the very low yields in this field, the toxaphene treatment was significantly poorer or almost so, than the other insecticide treatments only at the 5% level, except for treatment 6 where there was no significant difference between the two.

According to the data it appears that the wettable powder sprays of Sevin and DDT applied on a weekly basis did not need the addition of a sticker since equally good control was obtained without its use. The two 10-day schedule treatments (4 and 8) had the sticker added and there was as good control in these treatments as in the 7-day DDT or Sevin treatments. It would appear from these data that spraying intervals could be lengthened with the use of a sticker. However, this portion of the work needs further experimentation, particularly since there was no comparison tests between 10-day spray schedules with and without sticker. Although not significant, both the emulsion and wettable powder sprays of DDT at the 2-pound rate gave slightly better control than at the 1-pound rate.

The very low percentage of infested peppers in the DDT and Sevin treatments as compared with the checks, especially during the height of borer attacks (harvests 2, 3, 4 and 5), is substantial evidence of the excellent control achieved by these two insecticides. Considering that infested peppers usually result in a financial loss to growers, owing to rotting in the field or to rejection by inspectors prior to canning, it is encouraging to note that in over 17,000 peppers examined from the DDT and Sevin plots less than 1% of them were infested.

In addition to controlling the European corn borer in the DDT and Sevin plots an obviously high degree of protection was afforded against larvae of the tobacco hornworm, *Protoparce sexta* (Johan.), and the cabbage looper, *Trichoplusia ni* (Hbn.). Although no actual counts were made, high infestations of these two species were very common during the growing season in the check plots, and on several occasions hand picking of these pests was necessary in order to save these plants. Practically no infestations by these pests occurred in the plots treated with insecticides.

Populations of the green peach aphid, *Myzus persicae* (Sulz.), were very high during August and September, especially in the DDT treatments. Malathion (emulsion and wettable powder) applied at the recommended rates failed to control this aphid, suggesting possible resistance to this insecticide.

DISCUSSION AND OBSERVATIONS ON TIMING OF SPRAYS.—Field observations on the development of the European corn borer in field and sweet corn, potatoes and peppers were made during the season with reference to pupation, adult emergence and abundance, egg laying activity, and larval growth. Three periods of abundant adult activity and subsequent egg laying were noted. These were the second, third and fourth week of May, the first and second week of July, and the last week of August and first 2 weeks of September.

As may be noted in table 1, there was a relatively low infestation of borers (mature second generation) in the experimental plots at the time of the first harvest (August 17 to 20). However, in 1956, 1958 as well as in other fields under observation in 1959, infestations by second generation borers were also observed to be high. Despite the low second generation infestations in the experimental plots, it is logical to assume that spraying during a portion of July as well as from mid-August to early or mid-September is necessary for control of this borer in peppers.

The high infestations in the check plots for the second harvests (Aug. 27 to 31) in all three fields were mainly first and a few second instar larvae of the third brood. As would be expected, the following harvests showed infestations by progressively older borers. It is important to note, however, that first and second instar larvae were still present for the third and fourth harvests (September 8 and 15) indicating continuing attacks by newly emerged larvae from eggs laid over an extended period of time. These infestation data in the experimental plots correspond with observations on adult and egg-laying activity during this same period and again emphasizes the necessity for continuous protection with sprays during periods of adult emergence, egg deposition and hatching of new larvae.

Spraying did not appear to reduce adult populations in the pepper fields during the peak of adult activity in late August or early September. Moreover it did not seem to interfere with egg deposition, since egg masses, both hatched and unhatched, were noted on the underside of leaves and on pepper fruit in the treated and untreated plots. In most cases when egg masses were observed directly on pepper fruit there were no indications that borers had entered the fruit. This indicates that upon emerging the young borers do not go directly into the fruit but that there is a short period of dispersal and possible feeding on foliage as is their habit on corn. Winter laboratory studies, where adults were allowed to oviposit on caged pepper plants, tend to substantiate this observation. Upon hatching most of the larvae migrated over the plants and many were seen to lower themselves on silken threads onto lower leaves or to the soil in the pots. Foliage feeding by many of these young larvae prior to boring into stems and fruit was observed in the laboratory.

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Toxicological Aspects of 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) in Cows, Horses, and White Rats[1]

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ABSTRACT

Five horses exposed continuously to the vapors of 0.5 mg. DDVP/ft.[3] in a closed barn for 22 days displayed mild cholinesterase (ChE) depression in erythrocytes after 7 days followed by recovery to normal ChE concentrations at 11 to 22 days. The plasma was within normal limits of ChE activity throughout the test period. The concentration of DDVP (2,2-dichlorovinyl dimethyl phosphate) in the air varied between 0.24 and 1.48 microgm./liter or 1.4% to 8.4% of the daily DDVP dose.

Two cows suckling calves showed normal ChE levels in the erythrocytes while ingesting 200 p.p.m. DDVP daily. Severe depression occurred at 500 p.p.m. DDVP and a single dose of 27 mg./kg. caused cholinergic collapse with recovery. The ChE levels in the calves remained normal throughout the 78-day test. Female rats nursing litters were repeatedly intoxicated by oral administrations of 30 mg. DDVP/kg. The litters of these rats exhibited normal ChE levels in the erythrocytes and plasma as well as normal weight-growth curves.

Fresh liver macerates from different animals detoxified DDVP, according to bioassay with *Musca domestica* L., at a rate of about 500 microgm. DDVP/gm. of liver.

These findings clearly demonstrated that DDVP is completely detoxified in the animal body and does not accumulate nor store in animal tissues. The hazard of DDVP is related directly to the weight of acute intake rather than to repeated exposures or metabolic deviation of the compound.

The insecticidal behavior of DDVP (2,2-dichlorovinyl dimethyl phosphate) has been well investigated since its discovery (Mattson *et al.* 1954). Studies have been made on many different insects as shown in Chart 1. In this country DDVP is a toxicant in several poisoned baits and industrial insecticides, whereas overseas it is widely used in household space sprays at about 0.3% concentration in kerosene.

The toxicology of DDVP is still not widely understood, particularly as to its hazardous character (Hazelton 1955, Hayes 1960). The present study was made to determine the hazardous behavior of insecticidal concentrations of DDVP for mammals.

Durham *et al.* (1957) established the acute oral LD_{50} for male rats as 80 mg./kg. and 56 mg./kg. for females, whereas in respiratory chambers containing air concentrations of 31 to 118 microgm. DDVP/l., rats were killed (6/6) after 4.8 to 83.0 hours of exposure.

Rats exposed daily to the vapors of DDVP from doses of 2 mg./ft.[3] (Tracy *et al.* 1957) exhibited transitory cholinesterase (ChE) depression in erythrocytes. Normal concentrations of ChE were found after 5 to 6 weeks of continuous exposure. The levels of DDVP in the air of the chambers varied between 1.13 and 0.41 microgm./l.

Arthur & Casida (1957) found the LD_{50} intraperitoneal dose of DDVP in rats to be 6 mg./kg. They corroborated Van Die (1957) (see Metcalf 1959) that rat serum esterases reactivated erythrocyte ChE in red blood cell (r.b.c.) preparations previously treated with DDVP.

MATERIALS AND METHODS.—Test DDVP was prepared by redistilling technical DDVP at 1–2 mm./Hg. and collecting the distillates between N_D 20/20 1.4535–40. Assays by gas chromotography and infrared absorption indicated the pooled distillate was 100% DDVP (±1.5%).

For tests DDVP was diluted serially in distilled water or physiological saline. The dilutions were prepared by dissolving 1 or 5 gm. DDVP into 100 to 500 ml. volumetric flasks and then serially diluting 1 ml. until the desired concentration was obtained. Dilutions were made about 18 hours before use. The total acidities of the test solutions were usually measured shortly before and after use. With some variations it was found that 1 gram of DDVP in aqueous solution was neutralized by 5 mg. of NaOH (phenolphthalein). The acidity of aqueous DDVP increases measurably with time, but the increase is nearly 10 times slower at 1° to 5° C. than at 25° C. Decreased insecticidal activity of the DDVP acid solutions, however, was not detectable with bioassays until titrations were about 15 mg. NaOH/1 gm. DDVP.

Bioassay.—The laboratory colony of *M. domestica* used in these tests was originally from the Chemical Specialties Manufacturers Association (C.S.M.A.) non-resistant house fly strain. Test flies were 4 to 6 day olds of mixed sexes. About 60 flies were introduced into each 4-liter, wide-mouth, screw-capped glass jar. Food consisting of 20 ml. of C.S.M.A. formula soaked up in cotton balls was added to the jars. Test solutions or tissues were pipetted or placed on filter papers (4 to 8 cm. diameter) and then inserted into the jars, which were loosely sealed and incubated at 78° ± 3° F. Air was constantly exhausted from the top of the incubator. Actual kill counts were made after 20 to 21 hours. Assays of DDVP in distilled water showed that the sensitivity of the bioassay was about 0.5 microgram of DDVP.

The LD_{100} for house flies was 1.0 microgram of aqueous DDVP after 20 to 21 hours exposure. Each dilution of DDVP was usually tested in triplicate. An assay was considered valid if one jar of the replicates showed only 95% kill (57/60) or when not more than 7 flies in the 3 test jars were alive at 20 to 21 hours, providing the average kill in the 3 test jars at 0.5 microgram DDVP was not more than 60% after 20 to 21 hours. Control jars were considered valid when not more than 5% mortality (3/60) per jar occurred.

Cholinesterase.—The cholinesterase activity of erythro-

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CHART 1.—Reports on insecticidal behavior of DDVP.

INSECTS	KIND OF STUDY	AUTHORS
Beet leaf hopper	Laboratory	Peary 1959
Body lice and bed bugs	Laboratory	Fluno 1955, Cole & Burden 1956
Bot fly	Laboratory (horse tests)	Drummond *et al.* 1959
Cat and dog fleas	Laboratory and veterinary kennels	Fluno 1955, Lorber 1958
Cherry fruit fly	Laboratory	Frick 1957
Cigarette beetle	Laboratory	Tracy *et al.* 1957
	Tobacco warehouses	Tenhet *et al.* 1957, Tenhet 1958
Cockroaches	Laboratory	Acree *et al.* 1956
		Arthur & Casida 1957
	P.C.O. field study	Smith 1957
		Arbaugh 1959
Dog ticks	Kennels	Lorber 1958
Honey bees	Laboratory and field tests	Anderson & Atkins 1958
House flies and other flies	Laboratory and field tests	Mattson *et al.* 1955, Fluno 1955, Arthur & Casida 1957, Ihndris & Sullivan 1958, Kelsheimer 1958, Ogden & Kilpatrick 1958, Kilpatrick & Schoof 1959, Metcalf *et al.* 1959, McDuffie 1959
Mealybug	Hot house ornamentals	Kelsheimer 1958
Mediterranean fruit fly	Field (bait traps)	Steiner 1957, Gertler *et al.* 1958
Midges	Lakeside field tests	Hilsenhoff 1959
Mosquitoes	Laboratory	Fluno 1955, Mathis *et al.* 1959, Gahan & Noe 1955
Mulberry pyralid, silkworm tiger moth	Laboratory	Kuwana 1955
Phorid fly	Commercial mushroom cultures	Stoller 1957
Tobacco hornworm	Laboratory	Guthrie *et al.* 1959

cytes and plasma was measured according to Michel (1949). The system was modified so that 2 ml. of solution were used for pH determinations and 0.04 ml. rather than 0.02 ml. of erythrocytes were used (Durham *et al.* 1957).

Blood Samples.—Blood samples from the horses and cows were taken from the jugular vein. About 2,000 units of sodium heparinate in physiological saline were shaken in the tubes or syringes before the bleedings. Rats were bled by cardiac puncture. Clean but not bacteriologically sterile technique was used.

DDVP Content of Air.—To determine the concentrations of DDVP in air, samples of air were drawn by vacuum pump through a glass column containing 20 grams of Celite 545 (*ca.* 8×1 inches). The airflow was 6 liters/minute and 540 liters (90 minutes) were usually collected. The charged Celite 545 was extracted with 100 ml. of CS_2, separate 10-ml. fractions being gently pressed into the column with 1 to 2 p.s.i. air, excepting for the first two fractions which were absorbed by gravity. After the last fraction was absorbed, air pressure was applied to remove all but about 15 ml. of CS_2. The pooled CS_2 eluates were evaporated at −45° under high vacuum to near dryness. This concentrate was made up quantitatively in CS_2 to 2 ml. or 5 ml. and analyzed for DDVP content in a 3-mm. NaCl cell by the Perkin-Elmer Model 21 infrared spectrometer. The absorbency band at 9.55 microns for P—O—C groups was quantitative under the conditions of this analysis to ±1.5%. It was sensitive to not less than 20 microgm. DDVP/ml. Related compounds or degradation products of DDVP interfering with the analysis were strictly avoided or accounted for.

In Vitro Tissue Tests.—Autopsies were performed on the rats, cats and chickens with chemically clean but not sterile techniques. Dissections were attempted to minimize contaminations from adjacent tissues and blood. The fresh beef and pig livers were obtained from a private abbatoir.

The muscles and brains excised for *in vitro* detoxication tests were thoroughly cut with scissors immediately after dissection and macerated in a mortar. The livers were also cut into small pieces and then passed through a hand meat grinder. The macerated tissues were weighed into sterile test tubes and mixed with an equal weight of physiological saline to which a few drops of toluol was added, unless they were autoclaved (15 lb. steam pressure). The resultant preparations were just flowable. DDVP was pipetted into each tube to the appropriate concentration in volumes not exceeding 0.2 to 0.5 ml. of saline. The test tubes were plugged with sterile cotton and incubated at 37° C. for 18 hours in an air oven. Bacterial counts were not made although a few tubes showed probable anaerobic growths. These were discarded. Bioassays were made by discharging the preparations after incubation onto filter papers and testing them as described above.

RESULTS.—*Effect of DDVP on Horses in a Closed Stable.*—The test horses remained indoors throughout the experiment. The stable contained four box stalls on each side of the barn, separated by a central concrete walkway. One window was in each stall and a ventilator from each corner stall passed through the hayloft from the ceiling to the roof. The four ventilators and two windows in opposite corner stalls were left open during the test.

After cleaning, the central walkway was sprinkled daily with about 1 gallon of water containing 6 gm. of DDVP. The volume of water just wetted the walkway. The dose of DDVP was 0.5 mg./ft.^{3} for the 12,000 ft.^{3} in the stable. The sprinkled solutions approximated 0.16% DDVP.

In table 1 data show that the concentrations of DDVP in the air was highest 2 to 3.5 hours after application, and decreased nearly 75% within 12 to 13.5 hours. Although the lowest daily concentration occurred 22 to 24 hours after application, it was about 26% greater at 22 days than at 11 days. This probably indicates that absorption of DDVP occurred primarily on the concrete walkway, delaying the volatilization of the separate DDVP doses,

Table 1.—Concentrations of DDVP in the air of a closed stable treated daily with 0.5 mg. DDVP/ft³.

Days Stable Treated	Air Sample Hours After DDVP Applied	Air Temperature (° F.)	Concentration DDVP: Micrograms/l. of Air	Concentration DDVP: Micrograms/Cu. Ft.[a]	Per Cent DDVP[b] in Air Y/L = 17.7
11	2– 3.5	68	1.390	39.3	7.8
11	12–13.5	72	0.357	10.1	2.0
12	22–24	70	0.240	7.0	1.4
22	2– 3.5	78	1.480	42.0	8.4
22	22–24	72	0.350	9.9	1.9

[a] Calculated as 28.3 liters per cu. ft.
[b] Theoretically, 17.7 micrograms DDVP/l. would be in the air if 0.5 mg. DDVP/cu. ft. were completely volatilized.

but causing a greater concentration of DDVP in the air with succeeding treatments. The data compare favorably with the 1957 study of Durham *et al.* (1959) for tobacco warehouses treated with 0.5 mg. DDVP/ft.³, but are higher than those found in their 1956 study and by Tracy *et al.* (1957) in steel chambers.

The cholinesterase levels of erythrocytes and plasma for the horses are shown in table 2. When DDVP treatments began, the control horses were removed to a stable about 300 feet distant from the test stable. Horses Nos. 3, 4 and 5 showed distinct ChE depression in erythrocytes after 7 days' exposure (10 test days), whereas No. 1 showed depression after 4 days' exposure (7 test days). No. 2 did not show any deviation from normal ChE concentrations. Recovery apparently was rapid since the levels of ChE were normal for all the test horses at 11, 15 and 22 days. No deviation from normal ChE levels occurred in the plasma and the animals did not manifest any clinical symptoms during the test period. The transitory ChE depression in erythrocytes conformed with earlier findings in white rats exposed to DDVP vapors (Tracy *et al.* 1957, Durham *et al.* 1957).

Durham *et al.* (1959) observed that little anti-ChE activity occurred in the erythrocytes and plasma of monkeys and human volunteers exposed to vapors of DDVP from doses of 2 mg./ft.³, or less, although some inhibition was obtained at doses of 4 mg. and 10 mg./ft.³. Monkeys continuously exposed for 42 days to vapors from 2 mg. DDVP/ft.³ showed ChE depletion in erythrocytes, but the ChE inhibition at 22 days was indefinite. The pattern of the plasma esterases was similar except to a lesser degree.

Total erythrocyte and leucocyte counts of the test horses taken on each blood sample were well within normal range and indicate that physiological stresses during the DDVP treatments and the long period of continuous stabling did not effect them.

Effect of DDVP on Lactating Cows and Suckling Calves. —Two young milch cows (Nos. 07 and 23) were fed DDVP in their daily grain rations. They received 5-lb. portions twice daily and the DDVP was mixed into each portion just prior to feeding. This system worked well through 200 p.p.m. At 500 p.p.m. the cows refused the diet. Thereafter DDVP was administered orally in gelatin capsules twice daily. Cow No. 07 was pregnant and suckled a newly born bull calf (No. 96), whereas Cow No. 23 suckled a heifer calf (No. 95). Two grown heifers (Nos. 94 and 68) received the same grain rations, but no DDVP.

Cholinesterase measurements are reported in table 3 only for the erythrocytes, since repeated analyses showed that the plasma esterases varied so markedly as to be unreliable. Hermenze & Goodwin (1959) found no ChE

Table 2.—Cholinesterase levels of erythrocytes and plasma from horses in closed stable treated daily with 0.5 mg DDVP/ft.³.

Total Days Horses in Stable	No. Days Horses Exposed to DDVP	Accrued DDVP (Mg./Ft.³)	ChE	Test Horses[a] 1	2	3	4	5	Control Horses (No DDVP) 6	7	8	9	Averages TH[b]	CH[c]
				Δ pH/Hour										
1	0	0.0	E[d]	0.19	0.15	0.19	0.17	0.21	0.19					0.18
			P[e]	.75	.90	1.07	1.16	.76	.71					.89
3	0	0.0	E	.21	.20	.25	.20	.23	.23					.22
			P	.48	1.42	.55	1.25	.89	.66					.87
7	4	2.0	E	.13	.16	.24	.24	.21	.22				0.20	.22
			P	.80	1.14	.80	.48	.76	.42				.80	.42
10	7	3.5	E	.14	.18	.14	.12	.14	.18				.14	.18
			P	.61	.42	.90	1.04	1.62	.76				.92	.76
14	11	5.5	E	.27	.26	.26	.23	.24	.28				.25	.28
			P	.67	.80	1.34	1.10	.97	1.21				.97	1.21
18	15	7.5	E	.17	.16	.21	.15	—	.15	0.16	0.18	0.22	.17	.18
			P	.70	.64	.89	1.42	—	.87	1.31	.95	1.09	.91	1.05
25	22	11.0	E	.15	.21	.21	.20	—	.20				.19	.20
			P	1.12	.78	.68	.68	—	.68				.82	.68

[a] Horses Nos. 1, 2, 4, 6, 7, 8 and 9 were thoroughbred geldings. No. 3 was a mare and No. 5 was an old pony gelding.
[b] TH = Test horses.
[c] CH = Control horses.
[d] E = Washed erythrocytes.
[e] P = Plasma.

activity in bovine plasma for acetyl choline or butyryl choline. The erythrocyte ChE was not depressed in Cow 23 by 200 p.p.m. DDVP or a total of about 11 gm. accrued DDVP when fed over a period of 32 days. Cow 07 exhibited depression to the minimum limit of normal activity at 100 and 200 p.p.m. DDVP. The two suckling calves showed normal concentrations of ChE in the erythrocytes.

The ChE levels of erythrocytes of both milch cows were depressed severely by 500 p.p.m. or a dose of 4.5 mg. DDVP/kg. It is significant, however, that neither animal showed further ChE depletion of erythrocytes as doses increased to 2,000 p.p.m. (18 mg./kg.). Each cow ingested about 145 grams of DDVP during 30 days. The animals were healthy and produced milk to satisfy their rapidly growing calves.

The erythrocyte ChE activity of the suckling calves was at the lower limits of normal enzyme variations as it was also for control heifer No. 94. The heifer calf (No. 95) inadvertently ate some of the grain containing 500 p.p.m. DDVP when this diet was offered to the cows. A distinct reduction of ChE in the erythrocytes promptly resulted. On the other hand, recovery to normal concentrations followed within 18 days although the calf continued to suckle Cow 23 who constantly ingested high doses of DDVP.

At 70 test days, 3,000 p.p.m. or 13.5 gm. of DDVP (27 mg./kg.) was administered to Cow 07 and 2,000 p.p.m. or 9.0 gm. of DDVP (18 mg./kg.) to Cow 23 in a single dose. The latter animal reacted normally, whereas Cow 07 displayed symptoms of intoxication about 20 minutes after ingesting the DDVP. Within 45 minutes she collapsed with cholinergic symptoms, exhibiting copious salivation, tremors of the extremities, uncoordinated muscular activity, convulsions and diarrhea. She was always conscious and did not abort. An hour after collapsing she was quiescent but too weak to rise. Within 3 hours she was standing. The veterinarian administered therapeutic atropine sulfate. The animal walked to the barnyard and at the evening meal suckled her calf (96) as usual, although lactation was minimal. The cow aborted 6 weeks after shock, but specimens were not obtainable for analysis.

The oral shock dose of 27 mg. DDVP/kg. for Cow 07 agrees well with the shock dose of nursing white rats (30 mg./kg.). In the latter animals the oral shock dose is about 50% of the acute oral LD_{50} for female rats (62 mg./kg. $\pm$ 8 mg.). These data conform with the finding of Radeleff & Woodard (1957) that 25 mg. DDVP/kg. orally are cholinergic for sheep and the observations of Drummond *et al.* (1959) that 50 mg. and 25 mg. of oral DDVP/kg. are toxic for horses. On the other hand, the former workers reported that 10 mg. of oral DDVP/kg. was toxic for calves which differs significantly from the above value of 27 mg./kg. Furthermore, in the present tests 9 mg. DDVP/kg. caused no clinical symptoms in Cows 07 and 23 although their erythrocyte ChE was distinctly depressed (table 3).

Bioassays of Milk, Urine and Feces.—Milk samples were collected from Cows 07 and 23 each time the animals were bled. About 25-ml. portions of the milk were soaked into balls of cotton 3 to 4 hours after collection and inserted into 4-l. jars where house flies could feed on them. No significant kills of the flies were caused by the milk samples after 20 hours exposure. On the other hand, titrations of DDVP in milk showed that 0.5 micrograms of DDVP in 3 ml. caused 100% kill in 20 hours. It was evident from these data that the milk of Cows 07 and 23 contained less than 0.17 micrograms of DDVP/ml., and that DDVP would have been detected by this procedure if present in the milk at a concentration of 0.08 micrograms/ml.

Samples of urine and feces were collected from Cows 07 and 23 at 61 and 67 test days when they had ingested 4.5 gm. and 9.0 gm. of DDVP daily for 10 and 8 days, respectively. House flies were exposed for 20 hours to 25 ml. of urine soaked into cotton balls and also to 3 ml. of urine absorbed on filter papers. No significant kills were obtained. Freshly deposited fly eggs were inserted into 100-gm. samples of the wet feces, and larvae developed normally. Few larvae pupated probably owing to the high liquid content. Titrations with DDVP were not made in feces and urine. However, 50 micrograms of DDVP/gm. of feces prevented larval development of freshly deposited fly eggs.

Effect of Dermal Applications of DDVP on Cows.—The two heifers, Nos. 94 and 68, were washed daily with either a 1% aqueous solution or emulsion of DDVP or a 10% suspension of DDVP in water. About 6 fluid ounces (180 ml.) were applied to the back, front shoulders and upper back portions of the neck with a sponge.

The animals were restrained in their stanchions 5 to 6 hours after treatment to avoid ingesting DDVP by licking. About 90 ml. of the 10% aqueous mixture of DDVP was applied. The heifers were bled in the usual way. Two Guernsey heifers were used as controls.

The cholinesterase levels of the erythrocytes from Cows 94 and 68 remained at the lower limits of normal variations while 1.8 gm. of DDVP were applied daily for 21 days. For the first 10 days 1% aqueous solution was used, whereas 1% emulsion was applied for the next 11 days (fig. 1). The emulsion was prepared by diluting a concentrate containing 50% DDVP, 30% perchlorethylene and

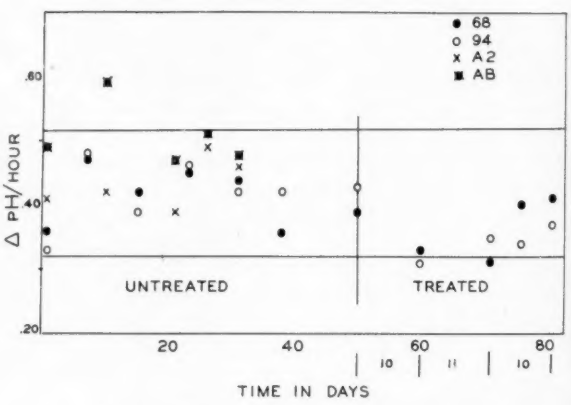

FIG. 1.—The ChE levels of erythrocytes in Holstein heifers Nos. 68 and 94 sponged daily with aqueous DDVP. The Guernsey heifers A2 and AB were controls for the treatment period. Treatments were 1% DDVP solution for the first 10 days, 1% emulsion for 11 days and 10% DDVP in water suspension for the last 10 days.

Table 3.—Cholinesterase levels of erythrocytes from milch cows ingesting DDVP daily and their suckling calves.

TEST PERIOD		DOSES OF DDVP INGESTED				MILCH COWS[a]		CALVES[b]		CONTROLS[c] NO DDVP	
								96	95		
Total Days	Days per Each DDVP Diet	P.p.m. by Weight[d]	Daily Dose in Gm.	Mg./ Kg.	Accrued DDVP (Gms.)	07	23	07	23	94	68
						pH/Hr. for Erythrocytes					
0	0	0.0	0.0	0.0	0.0	0.51	0.51				
4	4	0.0	0.0	0.0	0.0	.32	.35	0.38	0.31	0.33	0.36
7	3	0.0	0.0	0.0	0.0	.48	.45	.45	.45	.43	.47
16	9	1.2	0.005	0.01	0.05	.38	.32	.38	.30	.39	.42
24	8	20.0	0.090	0.18	0.78	.41	.45	.44	.47	.45	.45
32	8	100.0	0.454	0.91	4.41	.30	.35	.41	.32	.42	.44
39	7	200.0	0.910	1.8	10.78	.29	.39	.47	.48	.41	.49
51	12	500.0	2.275	4.5	38.08	.07	.06	.38	.18	.43	.49
61	10	1,000.0	4.540	9.0	83.48	.06	.06	.36	.28	.32	.33
69	8	2,000.0	9.000	18.0	155.48	.05	.09	.34	.31	.39	.47
70	1	2,000.0	9.000	18.0	164.48		.05		.33	.34	.40
70	1	3,000.0	13.500	27.0	168.98	.00		.32			
78	8	0.0	0.0	0.0	0.0	.01	.10	.39	.30	.37	.31

[a] No. 07 Holstein, pregnant, weighed 1,106 lbs. No. 24 Holstein weighed 1,160 lbs.
[b] No. 96, bull calf suckling Cow No. 07. No. 95, heifer suckling Cow No. 23.
[c] Holstein heifer No. 94 weighed 550 lbs. and No. 68 weighed 650 lbs.
[d] P.P.M. of DDVP in 10 lb. of grain diet by weight.

20% Triton X-155 in water. When 9.0 gm. of DDVP were applied daily as a water suspension for 10 days (70 to 80 days), no ChE inhibition of the erythrocytes occurred and the levels actually increased toward the higher normal concentrations. These data indicate that dermal absorption of aqueous DDVP *per se* was not toxicologically significant in the bovines. On the other hand, white rats treated with 1% aqueous DDVP over a 10-cm.^{2} clipped surface on the front shoulders and top of the neck exhibited a mild transitory ChE depression, whereas a severe ChE depression of erythrocytes and plasma was caused by applying aqueous DDVP containing a polyoxyethylene solubilizer. This result conforms well with the finding that DDVP in xylene is readily absorbed by the skin of rats (Gaines 1960).

Effect of Oral DDVP Doses on Lactating Rats Suckling Litters.—Seven rat mothers and their litters, 1 to 12 days old initially, were placed in separate cages and DDVP was administered periodically to each mother by stomach tube. The mothers were placed singly in separate cages containing clean sawdust for about 2 hours after each oral dose, and, if necessary, were washed before being returned to their respective litters. The animals were lightly anaesthetized with ether when the stomach tube was introduced and usually became conscious simultaneously with completion of the operation. The DDVP was in distilled water in concentrations of either 1 mg. or 10 mg./ml. The young rats nursed exclusively for about 21 days (*ca.* 40 gm. wt.). after which they would both eat and suckle. They were weaned about 35 days of age (*ca.* 90 gm. wt.).

Although doses of 10 and 20 mg. DDVP/kg. did not cause any symptoms of poisoning in the rat mothers or litters, the ChE levels of the erythrocytes of the mothers were depressed nearly 50%, whereas the plasma esterases were less effected (table 4). At the concentration of 30 mg./kg. DDVP caused shock symptoms in the rat mothers with varying degrees of severity after each oral dose. The cholinergic symptoms varied from irritability with salivation and nasal twitching or scratching to sialorrhea, pronounced exophthalmus with excessive lacrimation, diarrhea, muscle fasciculation, violent tremors, convulsions and death in extreme cases. The onset of symptoms was 10 to 20 minutes after DDVP ingestion and continued for 30 to 90 minutes. Upon recovery the rats behaved normally, showing no physical damage from the experience. At 40 mg./kg. the symptoms were invariably severe and all of the animals died in shock, although Rat No. 15 withstood 23 repetitions of this dose in 35 days before succumbing.

Both 30 mg. and 40 mg. DDVP/kg. seriously inhibited the ChE levels of the erythrocytes, but, curiously, not the serum esterases. In fact, in four animals (Nos. 5, 6, 9, 15) the serum esterases were higher than normal during the shocking doses. Although elevated ChE levels in erythrocytes and sera are not unusual for rats receiving prolonged subclinical doses of DDVP orally or by inhalation (2 mg./ft.^{3} or less), acute intoxications generally cause marked ChE depressions of the serum esterases and erythrocytes. Durham *et al.* (1957) found that DDVP added to the daily rations of rats caused an initial cholinesterase (ChE) depression in the r.b.c. and plasma, followed by a rise in 30 to 90 days to normal levels when the doses were 200 p.p.m. and lower. When the doses were 500 and 1,000 p.p.m. the AChE remained significantly less than normal at 90 days.

The mother rats received as accrued DDVP weights equal to nearly three to four times their LD_{50} (62 mg./kg. $\pm$8 mg.) during the test period, nevertheless they behaved normally and without detriment to the physiological development of their litters.

In figure 2 the bodyweight gains of the DDVP-treated litters are compared to litters of four normal mother rats. The plotted values are the average individual weights of each litter without sex differentiation. No significant deviation in the slope or range of the curves is apparent, excepting that the rates of growth are related to the number of individuals per litter. Curves 7 and 8 are normal litters with two and three members, whereas Curve 15 is a

Table 4.—Effects of oral doses of DDVP on rat mothers suckling litters. Physiological symptoms and blood cholinesterase levels.

Family No.	Total Days	No. Oral Doses	Weight of Mother (Gms.)	DDVP Doses of Mothers: Rate (Mgs./Kg.)	DDVP Doses of Mothers: Accrued DDVP (Mgs.)	Behavior[a] of Mothers	Rat Mothers Cholinesterase: Rbc	Rat Mothers Cholinesterase: Plasma
3	0	0	300	0	0.0	Normal	0.16	0.25
	4	2	320	10	6.4	Normal	.18	.18
	22	7	300	20	48.4	Normal	.10	.20
	61	21	275	30	222.7	Shock[b]	.07	.12
1[c]	15	0	230	0	6.9	Normal	.27	.16
	22	6	235	20	35.1	Normal	.11	.20
	47	12	230	30	119.1	Shock[b]	.07	.18
5	5	5	307	20	30.5	Normal	.10	.22
	25	13	290	30	143.6	Shock	.06	.14
	51	10	280	30	227.6	Shock[b]	.01	.44
6	0	0	270	0	0.0	Normal	.14	.16
	7	5	275	20	27.5	Normal	.10	.11
	25	12	250	30	117.5	Shock	.05	.13
	46	12	250	30	207.5	Shock[b]	.05	.31
9	0	0	290	0	0.0	Normal	.20	.36
	4	3	290	20	17.4	Normal	.14	.37
	25	12	280	30	118.2	Shock	.08	.23
	36	5	260	30	157.2	Shock	.05	.27
10	0	0	210	0	0.0	Normal	.32	.32
	28	14	220	30	92.4	Shock	.11	.16
	30	2	215	40	109.6	Shock[b]	.03	.15
15	0	0	232	0	0.0	Normal	.15	.23
	26	16	215	40	137.6	Shock	.04	.25
	35	7	215	40	197.8	Shock[b]	.08	.20

[a] Normal behavior implies lack of cholinergic symptoms from ingested DDVP.
[b] Died during shock or within 24 hours after last shock.
[c] Rat mother was pregnant while suckling a litter and was treated three times at 10 mgs. DDVP/kg. before second litter was born.

DDVP-treated litter with 11 members. The remaining weights of both normal and DDVP-treated litters fall between these extremes.

The average ChE levels of the erythrocytes and plasma of the DDVP-treated and normal litters are given in table 5. The plasma esterase levels are lower than those of the erythrocytes for nearly all of the young rats. This is the reverse for adult rats. On the other hand, all of the values for the litters are within the range of ChE concentrations of normal white rats. In table 6 data show that no significant deviations in ChE levels of the blood are apparent for different age groups of the young rats. It is evident from these tests that DDVP ingested by the rat mothers was not transported *per se* through their milk to the suckling young.

Bioassays of Tissues from Rats Ingesting DDVP.—Certain organs and tissues were dissected from rats killed with oral doses of DDVP. The animals had died either in DDVP shock or within 24 hours after the last dose. The specimens were placed on filter papers and inserted in 4-l. jars in which about 60 flies could feed at will for 20 hours. No other food was present.

Data in table 7 show the results with four different rats. Although esophageal and duodenal orifices of the stomachs were ligated to prevent internal leakage, this organ was highly toxic for the flies irrespective of the time of death. On the other hand, the liver was always nontoxic for flies. The brain apparently was toxic to flies when death occurred from between 1 to 2 hours to 12 to 16 hours after DDVP was ingested, but not after 23 hours. The heart and its blood, muscles and the small intestine were not toxic for flies. Adipose tissue also was not toxic, excepting for Rat No. 15. None of the rat mothers receiving comparably accrued weights of DDVP exhibited fat toxicity for flies.

Specific tissues were examined for detoxication of DDVP. Data are shown in table 8. It is evident that excised muscles, the small intestine and the brain do not detoxify DDVP *per se*, whereas excised liver does detoxify DDVP, the rate observed being 500 microgm. DDVP/gm. of liver at 20 hours incubation, 37° C.

In table 9 data show that fresh livers of several different animals, including beef, pig and poultry, detoxify DDVP. The rate varied somewhat. The slower rate of detoxication by fresh chicken liver undoubtedly reflects the higher avian susceptibility to DDVP (20 mg./kg., Durham *et al.* 1957). Equivalent weights of human liver were inconclusive since nearly 30% to 50% of the test flies survived at each test concentration. The results may have been caused by administrations of psychotherapeutic drugs prior to death.

DISCUSSION.—The prolonged oral intake (30 to 61 days) of DDVP by rats and two milch cows in nearly maximum sublethal doses well demonstrates the detoxica-

Table 5.—Levels of cholinesterase in erythrocytes and plasma of rat litters suckling DDVP-fed mothers and normal mothers.

Family No.	Rat Mother: DDVP Dose (Mg./Kg.)	Rat Mother: Accrued DDVP Ingested (Mgs.)	No. Rats per Litter	Age (Days)	Average Weight (Gms.)	No. Rats Tested	Δ pH/Hour Average of Tested Rats: Rbc	Δ pH/Hour Average of Tested Rats: Plasma	Δ pH/Hour Range: Rbc Min.-Max.	Δ pH/Hour Range: Plasma Min.-Max.
3	10	6.4	6	16	27	2	0.19	0.18	0.18–0.21	0.16–0.19
	20	19.0	6	27	64	3	.23	.18	.20– .27	.17– .18
	20	48.0	5	37	105	3	.22	.17	.20– .24	.16– .17
1	30	74.4	8	18	36	1	.20	.16	.20– .20	.16– .16
	30	112.0	8	25	55	4	.26	.19	.20– .29	.16– .23
5	30	82.0	5	20	49	3	.20	.21	.19– .21	.20– .24
	30	154.0	5	35	90	5	.25	.21	.18– .26	.20– .25
6	30	68.0	8	25	69	3	.21	.18	.19– .23	.16– .19
	30	120.0	8	35	107	8	.22	.17	.19– .26	.16– .18
9	30	61.0	4	24	55	2	.20	.18	.18– .21	.16– .19
	30	129.0	4	38	104	3	.22	.16	.22– .22	.14– .18
10	30	80.0	8	21	44	2	.24	.19	.21– .28	.16– .21
	40	109.0	7	27	61	2	.21	.23	.20– .22	.17– .28
15	40	119.0	11	21	33	2	.19	.22	.17– .21	.22– .23
	40	142.0	8	35	73	4	.23	.22	.22– .24	.20– .24
8	0	0.0	2	25	98	2	.26	.19	.26– .26	.16– .22
7	0	0.0	3	20	55	1	.16	.15	.16– .16	.15– .15
	0	0.0	3	36	118	3	.22	.18	.20– .24	.14– .24
4	0	0.0	5	32	70	5	.23	.17	.20– .27	.13– .21
2	0	0.0	8	21	39	2	.23	.20	.22– .25	.17– .22
	0	0.0	7	31	62	4	.24	.19	.23– .28	.14– .29

tion of DDVP *in vivo* in the animal body. This detoxication agrees with the observations that pentavalent phosphorus compounds are highly susceptible to metabolism and degradation in most biological systems (Metcalf 1959).

Detoxication is also shown by the nontoxicity of various rat tissues and organs, excepting the stomach, irrespective of the theoretical weight of accrued DDVP in the animals. In several rats the accrued weight of DDVP was nearly four times the LD_{50}. It is presumed from these observations that neither DDVP *per se* nor a toxic derivative is stored *in vivo*.

That the primary site of detoxication is the liver seems clear from the quantitative behavior of fresh liver macerates excised from several different animals in contrast to the inactivity of muscle, intestinal and brain macerates. Although the relatively high minimum concentra-

Table 6.—Comparison of blood cholinesterase levels of rat litters by age.

Eating Habits of Litters	Age in Days	DDVP Dose of Mother (Mg./Kg.)	No. Rats Tested	Δ pH/Hour Average for Tested Rats: Rbc	Δ pH/Hour Average for Tested Rats: Plasma	Δ pH/Hour[a] Difference Between Maximum and Minimum: Rbc	Δ pH/Hour[a] Difference Between Maximum and Minimum: Plasma
Nursing only	16	10	2	0.19	0.18	0.03	0.03
	18–21	30	6	.20	.19	.09	.08
	18–21	40	2	.19	.22	.04	.01
	18–21	0	3	.20	.18	.09	.07
Nursing and eating	24–31	20	3	.23	.18	.07	.01
	24–31	30	9	.22	.18	.05	.04
	24–31	40	2	.21	.23	.02	.11
	24–31	0	6	.25	.19	.05	.10
Weaned	32–38	20	3	.22	.17	.04	.01
	32–38	30	16	.23	.18	.05	.07
	32–38	40	4	.23	.22	.02	.04
	32–38	0	8	.22	.17	.06	.09

[a] Values valid to pH 0.04.

Table 7. Toxicity to *M. domestica* L. of Fresh Tissues from rats killed with oral doses of DDVP.

Rat No.:	K5[a]	15	16	K7[a]
Sex:	Male	Female	Female	Male
Dose of DDVP, mg./kg.	60	40	40	60
No. oral doses:	4	23	10	3
Mgs./doses:	2.1	8.6	6.6	2.0
Accrued DDVP, mgs.:	84	198	76	60
Hour death after dose:	23	16	1–2	1–2
Tissues	% Flies Dead After 20 Hrs. Exposure			
Brain	3.6	26.0	19.0	15.6
Heart	0.0	5.0	5.3	2.3
Liver	0.0	3.8	0.0	0.0
Stomach	100.0	100.0	100.0	100.0
Small intestine	0.0	6.2	8.1	4.0
Muscle[b]	9.0	7.1	4.3	8.3
Fat[c]	6.6	35.0	2.4	8.1

[a] The rat was administered DDVP orally twice daily at 9 A.M. and 4 A.M. unless sick.

[b] Muscle samples were pooled and included parts of the *longissimus dorsi, deltoid*, fore *biceps*, hind *biceps, gluteus maximus, femoris* and their associated muscles.

[c] Adipose about the kidney and groin.

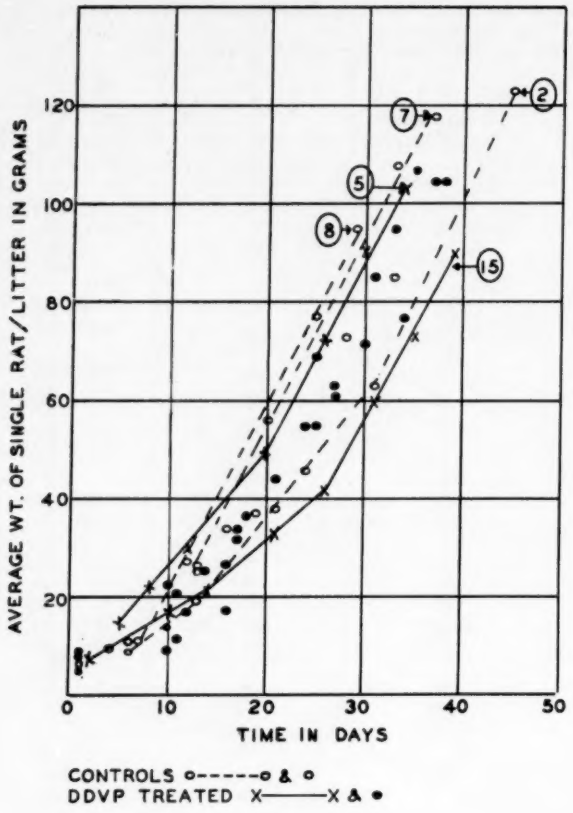

FIG. 2.—The weight-growth curves of rat litters suckling mothers ingesting DDVP and normal mothers. The values are the average weight of the individuals in each case.

tion of DDVP (250 microgm.) used in the tests may have masked the metabolic action of the latter tissues, the comparative activity of the liver clearly indicates its importance. Furthermore, the detoxication proceeds as a first-order reaction, characteristic of esterase/P inhibitor hydrolysis and agrees with the reported liver phosphatase activity for neurophosphates (Metcalf 1959). Arthur & Casida (1957) found that human sera decomposed DDVP, about 10% in 4 hours, but that no acid formation was detected in excised rat livers treated *in vivo* or *in vitro* with DDVP and suggested that the inhibition of DDVP resulted in the formation of the inactive enzyme dimethyl phosphoryl ChE.

The failure of fresh rat brain macerates to inhibit DDVP strongly suggests that AChE fixation in mammals is only one of several equally important detoxifying systems. This agrees with the findings that some neurophosphates (DFP) inhibit a wide range of enzymes (Heinicke & Mori 1959).

The lack of a toxicant for *M. domestica* in the milk of lactating rats and cows demonstrates that DDVP degradation was rapid and complete. The sensitivity of the bioassay was in the order of 0.01 microgm. DDVP/fly/20 hours exposure, which agrees with the established lethal

Table 8.—Bioassay with *M. domestica* of excised rat tissues incubated with DDVP.

	Weight of Tissue in Grams[a]														
	0.5				1.0				2.0				4.0		1.0
	DDVP (Mg.)														
Tissues	0.25	0.50	1.0	2.0	0.25	0.50	1.0	2.0	0.25	0.50	1.0	2.0	0.25	2.0	0.0
	Estimated Per Cent Flies Alive[b]														
Liver	95	0	0	0	95	95	0	0	95	95	95	0	95	95	95
Brain	0	0	0		0	0	0								95
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95
Small intestine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95

[a] Each weight of tissue was incubated with the different weights of DDVP for 20 hours at 37° C. The tissues were macerated in saline by hand so that a constant volume of 7 ml. was obtained, excepting the 4.0-gm. tests, in which the volume was 10 ml.
[b] After incubation, flies were exposed to the tissues in 4-l. jars for 20 hours at 23° C.

Table 9.—Detoxication of DDVP by excised livers from different animal species. Bioassay with *M. domestica*.

	Species of Animal																			
	Rat				Beef				Pig				Chicken				Cat			
	Weight of Fresh Liver (gm.)																			
DDVP (Micrograms)	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
	Estimated per cent kill of flies after 20 hours exposure																			
250	0	0	0	0	95	0	0	0	95	0	0	0	95	95	0	0	0	0	0	0
500	95	0	0	0	95	95	0	0	95	0	0	0	95	95	0	0	95	0	0	0
1,000	95	95	0	0	95	95	95	0	95	95	0	0	95	95	95	0	95	95	0	0
2,000	95	95	95	0	95	95	95	25	95	95	0	0	95	95	95	95	95	95	95	0
0		0	0			0	0			0	0			0	0			0	0	
	Autoclaved livers *Estimated per cent kill of flies after 20 hours exposure*																			
250	—	100	100	100	—	100	100	100	—	100	100	100	—	100	100	100	—	100	100	100
DDVP—Rate detoxified (mg.)		0.5/1,000				0.25/1,000				0.5/1,000				0.25/1,000				0.5/1,000		

doses for house flies (Arthur & Casida 1957, Metcalf *et al.* 1959, Mattson *et al.* 1955).

The portal of entry of DDVP is significant, the percutaneous route being nearly impervious to aqueous DDVP. By the respiratory route, however, horses displayed the mild transitory ChE depression in erythrocytes and plasma characteristic for insecticidal doses of DDVP (Durham *et al.* 1959, Tracy *et al.* 1957). The ChE depression observed in the erythrocytes of the animals was roughly proportional to the doses of ingested DDVP rather than progressively decreasing with time for a given dose, suggesting that a relatively constant rate of detoxication was maintained.

The behavior of DDVP in animals indicates that its real hazard is related more directly to the weight of acute intake rather than to accummulation in the animal body from repeated exposures to sublethal doses or to a metabolic deviation of absorbed or ingested DDVP.

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Toxicity of Organic Phosphorus Insecticides to Horses[1]

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Abstract

Tests were made on 48 horses to find the optimal dosage of some organic phosphorus insecticides for use against horse bots. (Gasterophilidae). The animals were treated with Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O*, *O*-diethyl phosphorothioate), dimethoate, DOWCO 105® (*O*-methyl *O*-(4-*tert*-butyl chlorophenyl) ethylphosphoramidothioate), DOWCO 109® (*O*-(4-*tert* butyl-2-chlorophenyl) *O*-methyl methylphosphoramidotioate), ronnel, Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), DDVP, and Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate). The insecticides were administered by stomach tube, by feeding, and by intramuscular injection. For 7 days until sacrifice, the horses were observed frequently and checked for whole blood cholinesterase activity. Although six of the animals showed signs of toxicity during the observation period, only one showed gastrointestinal congestion at necropsy. Fourteen horses owned by cooperating ranchers were treated with Dipterex in feed, with no harmful effect. The problems of determining poisoning from phosphorus insecticides are discussed.

This paper presents the toxicological observations of studies made in conjunction with Drummond *et al.* (1959), who tested new organic insecticides for the control of *Gasterophilus* spp. infesting horses.

Previous recommended treatments to rid horses of *Gasterophilus* larvae were oral treatment with carbon tetrachloride, carbon disulfide, or toluene. Todd *et al.* (1950, 1952) and Sinclair *et al.* (1953) found that toluene in doses of 0.1–0.2 ml./lb. body weight were effective in removing *Gasterophilus* larvae and produced no toxic reactions as did carbon tetrachloride. Graham & Alford (1951) reported that several chlorinated hydrocarbon compounds gave no control against horse bots. When carbon disulfide was given, they observed good bot control, but the treatment caused severe irritation of the gastric mucosa. Studies carried out in Germany by Bolle (1957) indicated that Neguvon® (*O*-*O*-dimethyl (2,2,2-trichloro-1-hydroxyethylphosphonate), also known as Dipterex® and Bayer L 13/59, to 30 to 40 mg./kg. was effective in removal of horse bots without producing symptoms of toxicity. Drudge *et al.* (1959) reported that dimethoate effectively removed bots at 40 mg./kg. in single doses, and that toxic symptoms appeared at higher levels of 60 and 80 mg./kg.

Animals, Materials and Methods.—Forty-eight horses, 2 to 17 years old, free of major disease, and in various condition, were purchased in groups of 6 from a horse slaughtering plant near San Antonio, Texas, and brought to Kerrville. The animals were numbered, weighed, and placed in individual pens. They were maintained on good quality prairie and alfalfa hay. The compounds were given in feed and by stomach tube directly into the stomach, and one compound was administered to one horse by injection into the muscular tissue.

Eight compounds were used in this series of studies. Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O*,*O*-diethyl phosphorothioate), dimethoate, DOWCO 105® (*O*-methyl *O*-(4-*tert*-butyl-2-chlorophenyl) ethylphosphoramidothioate), DOWCO 109® (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate), ronnel, Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), DDVP, and Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate).

For administration in feed, technical DDVP was dissolved in dilute alcohol and technical Dipterex was dissolved in water and mixed in 2 quarts of a commercial horse and mule feed. In most instances, the feed was readily eaten.

The compounds administered by stomach tube were placed in about 1 pint of water or dilute alcohol (dimethoate and DDVP) and pumped into the animal's stomach followed by two rinsings of equal volumes of water. Co-Ral, Dowco 105, Dowco 109, and Ruelene, each as a wettable powder containing 25% of the active ingredient, ronnel as ground-up commercially prepared bolluses, technical dimethoate, technical DDVP, and a soluble powder of Dipterex were used.

Dipterex was given intramuscularly as a 40% solution in diethyl succinate.

After treatment the horses were observed at frequent intervals day and night and were slaughtered 1 week after treatment. Their stomachs and intestines were examined for gross lesions that might result from the administration of the compounds.

An additional lot of 14 horses, 6 months to 10 years old, from six different cooperating ranches, was treated with Dipterex in doses of 37.5 mg./kg. mixed in feed. The animals' weights were estimated and the doses were measured accordingly. The horses were handled under practical field conditions and were observed by the owners for several days after treatment.

A record of whole-blood cholinesterase activity was kept for each horse for 7 days following treatment, with the exception of the 14 horses owned by cooperators. These horses received a control and one posttreatment blood sampling 1 day after treatment. Blood cholinesterase determinations were made by a modification of the electrometric method of Michel (1949).

Results.—Six animals that received Co-Ral, DDVP, Ruelene, and Dipterex exhibited toxic signs caused by the insecticides administered. No visible signs were seen in the animals receiving ronnel, Dowco 105, Dowco 109, or dimethoate.

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The animal that was treated with Co-Ral with a dose of 25 mg./kg. was most severely affected. He exhibited extreme abdominal pain evidenced by tensing of the abdominal muscles, rolling on the ground, and stretching. Borborygmi were audible several feet away. Diarrhea was profuse for several days, which added to an extreme weakness. No salivation was observed. Weakness continued until the animal was slaughtered. Blood cholinesterase was depressed to 20% of normal.

Two horses that were treated with DDVP by stomach tube—one at 25 mg./kg., and one at 50 mg./kg.—exhibited muscular fasciculations, abdominal pain, and general weakness. No salivation was seen. The animal receiving the low dosage showed mild signs, which appeared 15 minutes after treatment and lasted only a short time, and signs seen with the higher dosage were more severe and lasted for 2 days. Blood cholinesterase in both animals was depressed to about 55% of normal.

One horse treated with Dipterex in a dosage of 100 mg./kg. administered by stomach tube showed symptoms of mild abdominal pain and diarrhea. No salivation was observed. Blood cholinesterase was depressed to 7% of normal.

Two horses treated with Ruelene by stomach tube, one at 50 mg./kg., and one at 100 mg./kg., exhibited toxic signs. The animal receiving the low dosage was weak and exhibited incoordination and abdominal discomfort. No salivation was observed. Blood cholinesterase was depressed to 16% of normal. The horse given 100 mg./kg. showed very profuse salivation. The saliva was thick and hung in long strings, which at times almost touched the ground. Very profuse diarrhea and much abdominal discomfort were observed. These signs lasted 6 hours. The next day the animal was badly drawn and reacted in a manner suggesting impairment or distortion of its vision. Blood cholinesterase was depressed to 11% of normal.

The Co-Ral-treated animal showed marked congestion of the intestines and stomach. The other horses failed to show gross pathology. Maximum cholinesterase depressions for the horses are shown in table 1.

DISCUSSION.—It is interesting to note that signs of toxicity of these organic phosphorus compounds in horses are different from those seen in cattle, sheep, and goats. Signs seen in cattle and sheep with Dipterex or DDVP toxicity are excess salivation, difficult breathing, and stiff-legged walking. Co-Ral and Ruelene produce incoordination and weakness, with salivation and difficult breathing appearing inconsistently.

In five of six horses poisoned in these studies, no salivation was seen, and difficult breathing was not seen in any of them. In all cases the predominant symptoms were abdominal pain and general muscular weakness. It would be extremely difficult to distinguish between colic produced by phosphorus insecticides and colic produced by intestinal impaction or some other condition.

It appears that in the case of DDVP when mixed in feed, the cholinesterase depression is greater than when given by stomach tube. However, symptoms are observed when doses of 25 and 50 mg./kg. are administered by stomach tube, but symptoms do not appear when these same doses are given in feed. This fact may be due to a binding effect of the compound in feed, causing it to be released more slowly.

Table 1.—Whole blood cholinesterase activity (ChE) in horses treated with organic phosphorus insecticides.

COMPOUND	DOSAGE (MG./KG.)	ADMINISTRATION ROUTE	LOWEST ChE DEPRESSION (% OF NORMAL)	REMARKS
		Single Doses		
Co-Ral	25	ST[a]	20	Severe poisoning
Dimethoate	25	ST	100	No visible effect
	50	ST	58	No visible effect
Dowco 105	100	ST	42	No visible effect
Dowco 109	10	ST	32	No visible effect
	25	ST	31	No visible effect
	50	ST	18	No visible effect
	75	ST	25	No visible effect
	100	ST	32	No visible effect
	100	ST	51	No visible effect
	100	ST	19	No visible effect
Ronnel	110	ST	77	No visible effect
Ruelene	25	ST	56	No visible effect
	50	ST	16	Poisoning
	75	ST	39	No visible effect
	100	ST	11	Severe poisoning
DDVP	10	ST	58	No visible effect
	10	F[b]	42	No visible effect
	25	ST	56	Mild poisoning
	25	F	26	No visible effect
	50	ST	53	Poisoning
	50	F	15	No visible effect
Dipterex	10	ST	47	No visible effect
	10	F	69	No visible effect
	10	F	64	No visible effect
	10	F	—	No blood sample
	25	ST	34	No visible effect
	25	F	44	No visible effect
	25	F	37	No visible effect
	25	F	28	No visible effect
	25	F	26	No visible effect
	25	IM[c]	28	No visible effect
	37.5	F	55	No visible effect
	50	ST	21	No visible effect
	50	ST	37	No visible effect
	50	F	23	No visible effect
	50	F	32	No visible effect
	50	F	39	No visible effect
	50	F	33	No visible effect
	50	F	20	No visible effect
	75	ST	23	No visible effect
	75	ST	46	No visible effect
	75	F	15	No visible effect
	100	ST	7	Mild poisoning
	100	F	67	All of feed was not eaten
Dipterex[d]	37.5	F	37–62 (Avg.—48)	No visible effect
		Multiple Doses		
Dipterex	25[e]	F	28	No visible effect
	37.5[f]	F	20	No visible effect
	50[g]	F	13	No visible effect

[a] ST—stomach tube. [b] F—in feed. [c] IM—intramuscularly. [d] Treated 14 cooperator—owned horses. [e] 25 mg./kg. fed two times in 1 day. [f] 37.5 mg./kg. fed two times in 1 day. [g] 50 mg./kg. fed one time/day for 2 days.

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Long-Range Aerial Transport of the Harlequin Bug and the Greenbug into Minnesota[1]

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ABSTRACT

On May 4, 1959, the harlequin bug, *Murgantia histrionica* (Hahn), appeared in large numbers in a restricted locality in southern Minnesota. Fourteen days later an outbreak of the greenbug, *Toxoptera graminum* (Rond.), was discovered in the same general area. A study of weather records for the first 4 days of May showed that weather features particularly favorable for long-range aerial transport of insects could have enabled them to be carried to Minnesota from areas to the south and southwest, where they were known to be abundant. The possible prediction of future major outbreaks of the greenbug is discussed briefly.

During early May 1959, southwestern Minnesota was invaded by the greenbug, *Toxoptera graminum* (Rond.), and the harlequin bug, *Murgantia histrionica* (Hahn). The greenbug, while not a permanent resident of the State, is known to be a frequent immigrant from areas to the southwest, and occasionally it has increased to outbreak proportions after its arrival (Wadley 1931). In contrast, the discovery of the harlequin bug in the town of Slayton in southwestern Minnesota was a unique event. This species had never before been collected anywhere in the State. Because of this a brief account of its arrival is given here.

A greenhouse operator was covering cold frames in the late afternoon of May 4 to protect plants from a threatening storm, when he noticed very large numbers of flying insects in the yard. At first he thought they were honey bees, but unlike honey bees they soon settled on young cabbage plants in flats, where they immediately started to feed and oviposit. A careful survey made by personnel of the State Division of Plant Industry showed that the infestation was localized in an area about 1 square block to the east and including the place where they were first found. This site is designated by a large dot in Central Murray County in figure 1. Within a few days more harlequin bugs or reports of their being found were received from one locality to the east and several northeast of the original find. Much later in the summer harlequin bugs were collected north and northwest of what is presumed to have been their line of flight upon entering the State in May. The locations of these late reports, received in August, also are shown on the map in figure 1.

This account of the arrival of the harlequin bug in Slayton, Minnesota, suggests that we may have been fortunate enough to obtain a record of the day, and perhaps even the hour that the bugs terminated their flight from the southwest. We cannot be as sure about the time of arrival of its fellow travelers, the greenbugs. When they were first reported they already were present in abundance in several southwestern counties, (fig. 1), and were causing conspicuous damage to small grains. The date of their discovery was May 18, 14 days after the first harlequin bugs were seen. However, it is assumed that the principal greenbug invasion from the southwest probably occurred at the same time as that of the harlequin bug, because a large aphid population could have arisen from early established immigrants with the temperatures that prevailed during this 14-day period (Wadley 1931).

SOURCE OF INFESTATION.—As neither of these species is a permanent resident of Minnesota, and both are common pests south of lat. 40° N., the northern border of Kansas, it is pertinent to ask whence they came and by what means. Records obtained from the Cooperative Economic Insect Reports issued by the Plant Pest Control Division, Agricultural Research Service, U. S. Department of Agriculture show that the harlequin bug was present in abundance and was killing crucifers and causing considerable damage to ornamentals in Oklahoma and Texas during the week ending May 8. Damage continued to be reported from these States during the month of May. The records for the week ending May 29 indicate that unusually large numbers of harlequin bugs were present on weed hosts in Nebraska.

Dense populations of the greenbug also were reported early in the season from Oklahoma and Texas. Heavy infestations occurred in Tillman County, Oklahoma, during the week ending March 13, and heavy populations of winged forms were noted in Noble and Payne Counties during the week ending March 30. At the close of the week ending April 17, populations were declining in Oklahoma and the first reports of light to moderate populations were received from Kansas. Greenbugs continued to increase in abundance in Kansas, and by the last week in April they were increasing in abundance also in southwestern Missouri. During the following week ending May 8, populations were still increasing in Kansas, and a large proportion of the populations in both Kansas and Missouri had developed winged forms. Thus the stage was set in late April and early May with both the har-

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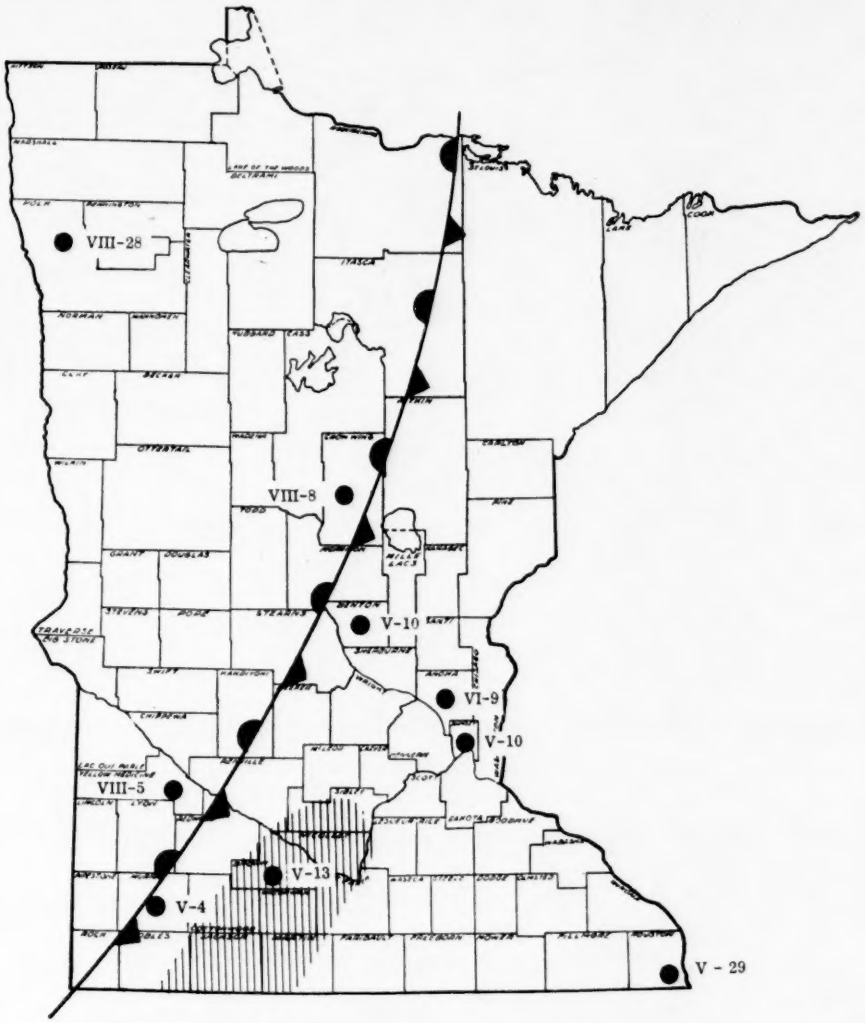

FIG. 1.—Position of front on the afternoon of May 4. Spots show localities and dates where harlequin bugs were collected. Shaded area shows distribution of greenbug when first detected.

lequin bug and the greenbug present in abundance in areas to the south and southwest of Minnesota.

METHOD OF MIGRATION.—The second part of the question stated earlier concerns the means of travel from the areas of known abundance, cited above, to southwestern Minnesota. Flight, aided by favorable wind circulation, is proposed as the simplest answer. This opinion is supported by the work of Wadley (1931). He states that prior to the discovery of the 1926 outbreak in Minnesota there were certain well-defined meteorological conditions that occur infrequently. These conditions were strong southwest winds that were sustained without interruption for 2- or 3-day periods. The winds were channeled from south of Oklahoma, across Kansas, and on northeastward into Minnesota. Wadley's description of weather conditions associated with the 1926 greenbug outbreak seems to satisfy the theoretical pattern required for long-range aerial transport of insects (Wellington 1945). According to Wellington there should be a high-pressure area which remains relatively stationary for several days, accompanied by strong winds that are maintained along a definite direction of circulation throughout the stationary period. Such conditions, he says, may occur in spring or autumn, and may well act to distribute numbers of a given species for hundreds of miles along a fixed track, thus insuring the establishment of the species in any suitable habitat along the wind path. With this information to serve as a guide the authors examined weather records assembled by the U. S. Weather Bureau for the first week in May 1959, the week during which it is assumed that the long range transport of the harlequin bug and the greenbug had occurred.

METEOROLOGICAL EVENTS.—The sequence of meteorological events leading up to the sighting of the harlequin bug on May 4 are illustrated in figures 2, 3, 4 and 5. According to Woffinden (1959), there was a temperature anomaly during the first week of May characterized by a strong east–west temperature gradient. Unseasonable cold weather prevailed over the western United States, while much above average temperature was reported in

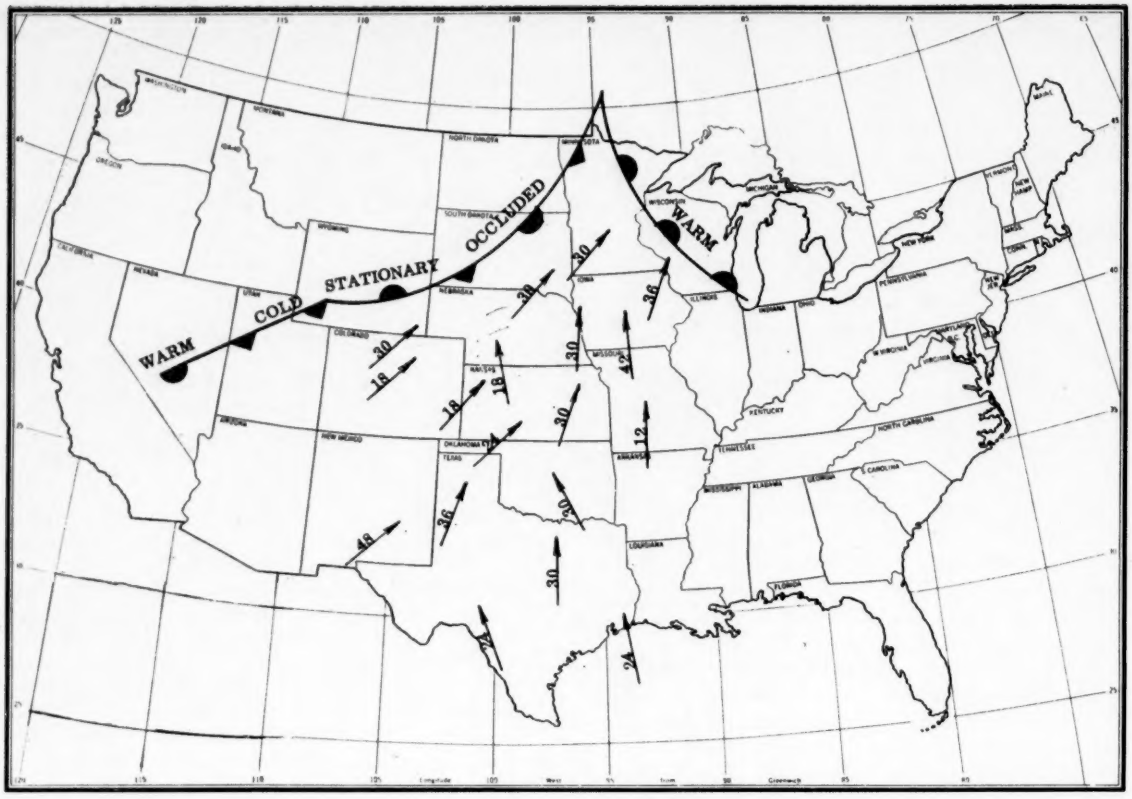

FIG. 2.—Position of front at noon of May 1. Arrows indicate wind direction and velocity in miles per hour at second standard level (between 200 and 1,200 feet above ground surface at weather station).

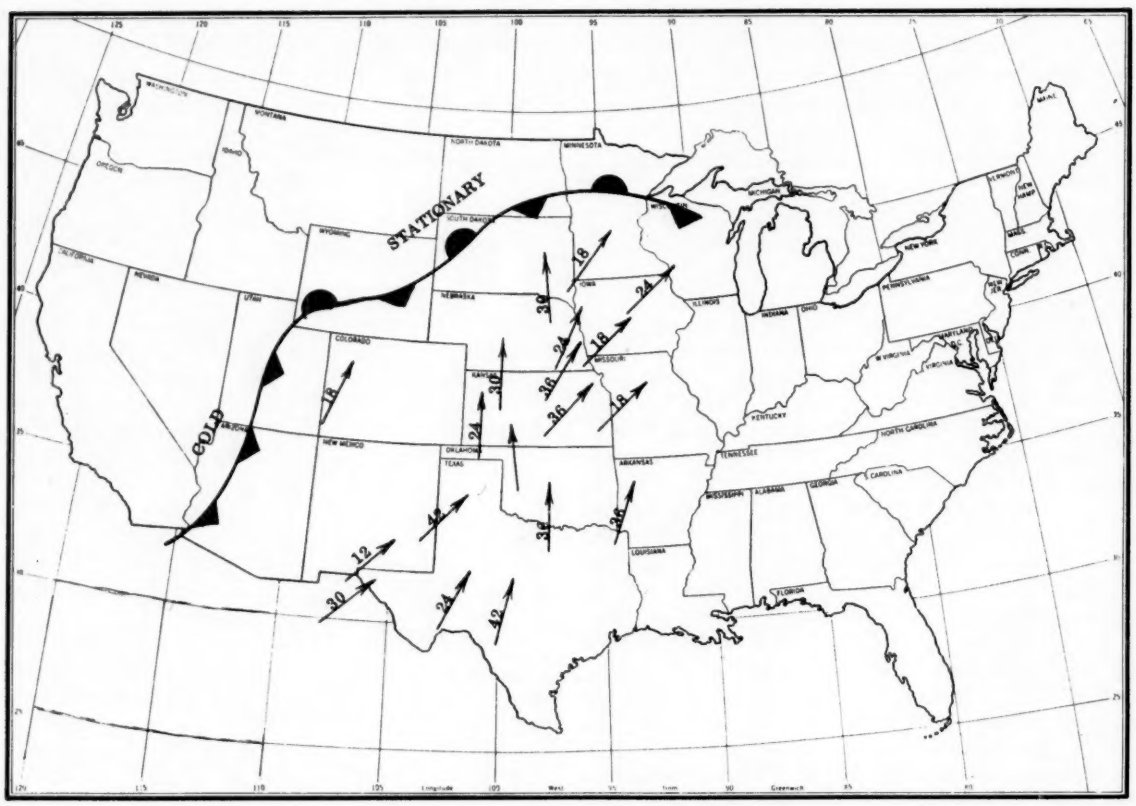

FIG. 3.—Position of front at noon of May 2.

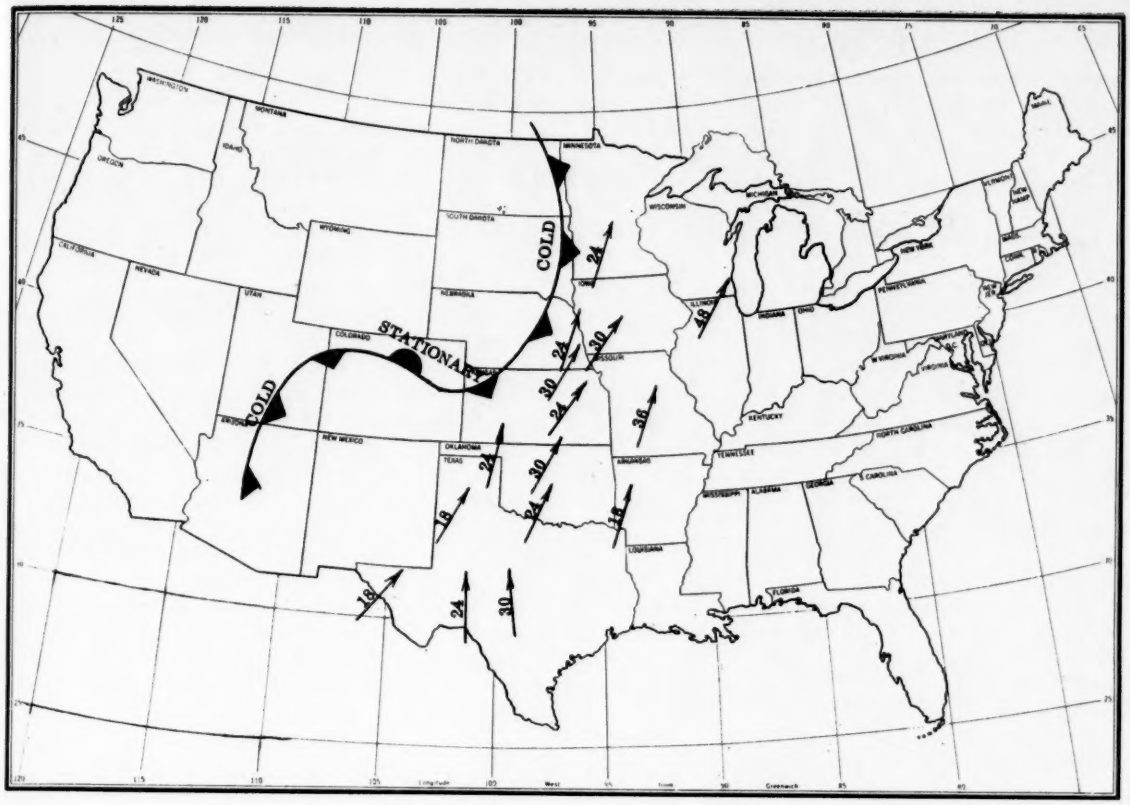

FIG. 4.—Position of front at noon of May 3.

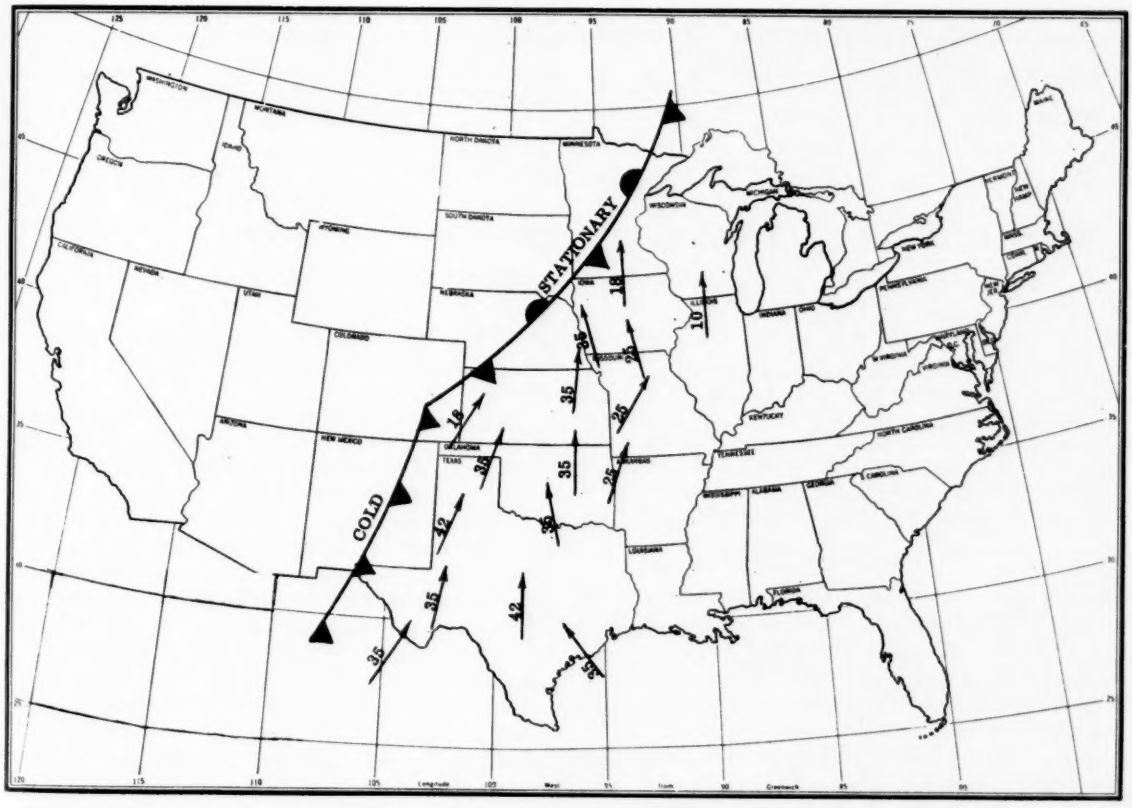

FIG. 5.—Position of front at noon of May 4.

the central Great Plains in advance of the frontal system shown in figures 2, 3, 4 and 5. A trough formed along the line of the frontal system, and a blocking effect amplified a ridge in front of the trough, thus advecting warm, moist air into the Mississippi Valley. Along the boundary between the contrasting air masses severe frontal weather developed with thunderstorms and considerable convective activity.

The frontal systems shown on the maps maintained a prolonged and relatively constant position throughout the 4-day period prior to the arrival of the harlequin bug in Slayton. A sustained circulation of strong southwest winds from Texas, across Oklahoma, Kansas, and eastern Nebraska was channeled along the frontal system at the same time. These winds were constant in direction from the surface to 18,000 feet. They were brisk from Texas to northern Iowa, but diminished in velocity in southwestern Minnesota by noon of May 4.

Thus the conditions for long-range transport of winged adult harlequin bugs and greenbugs, known to be abundant along the air-circulation path, fit the ideal pattern set forth by Wellington. Furthermore, the air turbulance and convective activity in advance of the frontal system could have been responsible for the insects' becoming airborne in the first place. Finally, the drop in wind velocity in southwestern Minnesota, which was most noticeable by noon of May 4 (fig. 5), may have permitted the airborne insects to descend into the area where they were seen first about 4:30 p.m. that afternoon. Harlequin bugs may have dropped out along the path of the winds before reaching Minnesota. This supposition seems evident from the fact that unusually large numbers were reported from Nebraska, and they also were found in Iowa (personal communication from H. M. Harris).

Mention was made earlier of reports that the harlequin bug had been found in August. The location of these finds seen in figure 1 shows them in positions behind the occluded front that lies diagonally across Minnesota. These bugs may have moved into western counties soon after they arrived in May and were not found until much later, or they may have been the adults of a second generation produced in Minnesota. The greenbug spread from the original heavily infested area (fig. 1) to be found later in nearly all counties in the southern two-thirds of the State.

PREDICTING OUTBREAKS.—The now well-established association of recognizable weather features with outbreaks of the greenbug in Minnesota suggests the possibility of predicting the development of major outbreaks in northern areas in the future. The first step would be to keep informed concerning the progress of greenbug development and numbers in areas in the South and Southwest. Then a daily scanning of weather maps during late April and May would make it possible to observe the development of the critical patterns of weather necessary for long-range aerial transport. Finally, whenever the required combination of weather features occurred, surveys could be conducted in small grain fields with a reasonable chance of detecting light populations in time to initiate control before extensive damage takes place.

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Effects of An Aerial DDT Spray on Aquatic Insects in Connecticut[1]

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ABSTRACT

Ten foot-square samples were taken at each of five streams before and after a woodland DDT spray was applied to check the qualitative loss of insect genera. Disregarding those genera that disappeared from the streams by reason of adult emergence only two insects disappeared from all the treated streams. However, individual streams varied considerably in their individual losses, ranging up to a possible loss of 42.8% of the genera in one stream.

With the increasing use of insecticides a corresponding increase of opposition has grown in the public print against their use because of their effect on wildlife. As DDT has been the principal insecticide used for spraying woodlands, most interest has centered on this chemical, and several studies have concerned this pesticide and its relation to wildlife. Rudd & Genelly (1956) have reviewed and summarized information on this problem. Graham & Scott (1959) in Montana; Kerswill *et al.* (1958), Ide (1957), and Webb *et al.* (1959) in New Brunswick; and Filteau (1959) in Quebec have made recent studies of the effects of aerial DDT sprays on aquatic organisms.

In order to gain an understanding of this problem as it particularly related to Connecticut conditions, studies were begun in the spring of 1959 to measure the consequences of DDT sprays on stream insects.

The principal reason for aerial spraying of woodlands in Connecticut is to control the gypsy moth (*Porthetria dispar* (L.)). Although the economic benefit derived from spraying probably only equals its cost (Turner 1958), there is little likelihood that this type of spraying will cease in the near future. Generally, however, large areas are not sprayed at any one time. The insecticide used is DDT in No. 2 fuel oil at the rate of 1 pound of DDT per acre applied by airplane. All treated areas mentioned herein were served in this manner. An excellent

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kill of the gypsy moth larvae was obtained in every instance.

METHODS.—An effort was made to sample streams that would be sprayed from their source to a point below the sampling station, to minimize the effect of insect drift from upstream. Because of the spotty spray treatment, this sampling was possible in only three of the four treated streams checked.

The stream listed as Hamden I was 3 feet wide at its widest point, narrowing down to 6 inches at small waterfalls. It had a rocky bottom with small pools occasionally as deep as 12 inches. A fairly rapid stream, it ran through open woods with small trees arching over the stream.

The stream Bethany I varied from 1 to 6 feet wide. Muddy-bottomed pools were interspersed with short riffles. Small trees and bushes overhung the stream bed.

The stream Bethany II also measured up to 6 feet across with long foot-deep pools separated by very short riffles. The brook had a bottom of moss-covered rock interspersed with sand and occasionally muddy banks. It was fairly open above.

Bethany III had pools up to 3 feet deep. Considerable difference was noted in water volume between spring and fall, with the water flowing between and over boulders. The banks of this rapid stream were heavily forested but the stream bed was so wide that the water ran in the open for considerable distances.

The check stream (Cheshire) had very long riffles over bare and moss-covered rocks. This brook averaged about 6 inches deep and was heavily shaded by the surrounding woods.

All the streams sampled were within 6 miles or less of each other. Two of the streams contained 38 genera of aquatic insects; two, 37 genera; and the remaining one, 34 genera. This similarity in the numbers of genera present was also tested by comparing the genera present in the check stream with those in the others. All four of the treated streams held 14 genera in common with the check. Only three genera were found in the check stream only. The other genera were found in one to three of the other streams.

No completely satisfactory method of quantitative stream sampling for aquatic insects has been developed. Not only is it difficult to reach many areas of the stream bottom, but a great natural variation in the fauna occurs at various places within the stream and at various times of the year (Badcock 1954). The commonest method of sampling, and the one used in this study, is by means of a Surber foot-square sampler. In order to obtain significant quantitative results, large numbers of samples are required. Needham & Usinger (1956) reported that even in a single riffle, 194 samples would be required to give significant figures on total wet weights and 73 samples would be required for total numbers. Although this work has been criticized (Macan 1958), it nevertheless remains the only systematic study yet made of the variability of the insect fauna within a stream. When it is considered that Needham & Usinger (1956) found it necessary to have at least two samples to pick up even the main orders of insects within a single riffle, it is clearly apparent that two samples cannot represent the species fauna of an entire stream, much less give an adequate basis for quantitative comparisons.

In the present study, 10 samples per stream were picked arbitrarily to measure the number of genera present. That this sample also was too small is indicated by the fact that at times genera were picked up in small numbers at later samplings that were not found earlier. To help guard against a supposition of loss of insects owing to the application of DDT, with the real cause the artifact of small sample size, only those insects were considered important to the study that reached a total of 5 specimens for the 10 samples in pretreatment counts. If insects were found in the posttreatment counts, even in numbers less than five, they were counted as escaping eliminative effects of DDT.

Generic and subgeneric names as used herein are those given by Usinger (1956) except for the Ephemeroptera, which follow Berner (1959) and Edmunds (1959).

RESULTS.—Table 1 shows the results with some of the insect genera present 1 week before and 1 week after spraying. If insects were found in the second sample that were not present in the first, they were considered to have survived the spray, because, with the exception of Bethany III and the check streams, all brooks had been sprayed from their source to a point below where the sample was taken. This repopulation, therefore, could not come from insect drift from an untreated headwaters. Those genera that were found only before spraying but in numbers less than five are noted in table 1 with the letter "A." This method can provide only evidence supporting other conclusions. For example, in the case of

Table 1.—Survival of some insect genera 1 week after an aerial DDT spray, based on 10 square-foot samples.

GENUS	STREAM				
	Hamden I	Bethany I	Bethany II	Bethany III	Cheshire (Check)
Ectopria	O	O	O	A	X
Promoresia larvae	X			X	X
Promoresia adults	X			O	X
Anchytarsus	X	X	X		
Rhyacophila	O	X	O		X
Lepidostoma	X	A	X		X
Neophylax	O	O	X	A	
Hydroptila	A	A	X	O	
Simuliidae	O	O	O	O	O
Chironomidae	X	O	X	O	X
Dicranota			X	X	X
Pseudolimnophila	X	O	X	X	X
Stenonema	O	O	X	A	X
Baetis	O		O	X	O
Habrophlebia	X	X	O		X
Paraleptophlebia	X	X	X	X	X
Ephemerella (*Ephemerella* s.s.)	X	X	X	X	X
Ephemerella (*Eurylophella*)	X	X	X	X	X
Ephemerella (*Drunella*)	X		X	X	X
Nemoura	X	X	X	X	X
Peltoperla	X	X	X	X	X
Isogenus		X	X	O	X
Isoperla	A	X	X	O	O
Acroneuria	A	O		A	X
Nigronia	X	X	X	X	X
Lanthus	X	X	X	A	X
Cordulegaster	A	X	X	A	

X = found in posttreatment counts.
O = found only in pretreatment counts, in numbers of five or more.
A = found only in pretreatment counts, in numbers of less than five.

Ectopria this loss is probably significant whereas it is probably not with *Lanthus*.

Definite evidence of the complete elimination of an insect from all the sprayed streams was present in very few cases. The coleopteron *Ectopria* of the family Psephenidae and *Rhyacophila fuscula* (Walker) of the trichopteran family Rhyacophilidae disappeared from the treated streams but not from the check stream. Other *Rhyacophila* survived in some streams but not in others.

In other cases, such as the Simuliidae, the emergence of adults prior to and during the 2-week test period removed the larvae from the check stream. *Baetis* appeared to have emerged also during the period of this study, as the nymphs were missing from posttreatment counts in both the sprayed and check streams.

Several other genera were apparently eliminated in some streams but not in others. Undoubtedly the type of stream and the nearby plant cover governed the amount of DDT available to the insects. Those insects that were especially hard hit by DDT sprays were *Rhyacophila*, *Neophylax*, *Hydroptila*, Chironomidae, *Isoperla*, and *Acroneuria*. In addition almost every genus listed in table 1 was reduced in numbers by the insecticidal spray.

As a group, the Megaloptera and Odonata appeared to be least affected by DDT. However, the number of these specimens captured was less in both counts than were the numbers of other insects. Coleoptera, other than *Ectopria*, seemed only slightly reduced by the spray. However, the larvae of *Promoresia* showed a different response than did the adults. The Trichoptera did not exhibit a consistent response to the insecticide in all the streams. It would appear that under the proper conditions caddisflies might suffer severe losses from DDT. The carniverous genera *Isoperla*, *Isogenus*, and *Acroneuria* of the Plecoptera suffered greater mortality than did the noncarniverous *Nemoura* and *Peltoperla*. This fact was probably a reflection of their habitats. The latter two genera are often found concealed in leaf packets, whereas the predaceous forms are more likely to be ranging freely over stones on the stream bottom. Furthermore, a carniverous species, by feeding on several herbiverous forms, will tend to concentrate the insecticide within its own body.

It may be noted that the Ephemeroptera as a group were not eliminated from the population. They, in fact, often suffered less of a numerical loss than did some of the other aquatic orders. This result was most noticeable in the genus *Ephemerella*. The genera *Stenonema*, *Habrophlebia*, *Ameletus*, and possibly *Epeorus* and *Baetis* were apparently more affected by the insecticide. That all species of mayflies are not particularly susceptible to DDT or that resistance may possibly develop is indicated by the presence of these insects in irrigation ditches in agricultural land that is treated heavily with insecticides.

Planarians and the arachnid *Limnochares* survived the treatment.

Table 2 gives a summary of the counts on all insect genera, except the Odonata, found within the streams.

More genera survived in the untreated check than in streams treated with the insecticide. It may be noted that four genera in the check stream did not reappear in the posttreatment counts and it might be presumed that these four genera emerged as adults and should not be counted in the treated streams. However, one of these genera appeared in no other stream and another was represented in the treated streams by pretreatment numbers of less than five specimens. Consequently, only two genera could be considered for certain to have been eliminated from the larval population of the treated streams by the emergence of the adults.

The minimum possible loss of genera occurred in Bethany II with 21.4%, which compared with a minimum possible loss of 15.4% in the untreated check. The maximum possible loss took place in Bethany I, with 63.6%. Because of sampling error in those genera captured in numbers less than five, the actual loss was probably much nearer the minimum than the maximum figure.

DISCUSSION.—A considerably greater qualitative loss occurred in the sprayed than in the unsprayed plots. This loss was not so serious as it might seem at first glance. In only the two cases mentioned above was an insect apparently eliminated from all the treated streams. In the numerous situations wherein a genus was apparently eradicated from one stream, there was some survival in nearby streams, even when they were treated also.

If an insect is eliminated from a stream, its reoccurrence depends on the means available to re-enter the barren section. Insect drift from upstream can very rapidly repopulate the lower reaches of a stream (Mueller 1954) at any season. Movement upstream by larvae is very slow (Harker 1953), when it exists at all. As demonstrated by Roos (1957), gravid females with mature eggs will move upstream to oviposit, and in most cases repopulation of the upper reaches of a stream will take place in the spring.

No good figures are available on all orders of aquatic insects as to the distance they will fly over land to repopulate new stretches of water. Roos (1957) indicated that some Trichoptera can extend their flight for 5 kilometers and Burks (1953) thought it possible that gravid females of the ephemerid *Tortopus primus* (McDunnough) might fly as much as 20 miles. Frison (1935) collected Plecoptera 5 miles from the nearest stream. It would appear that there are enough strong fliers in all the aquatic orders to repopulate nearby streams from which all insects have been removed. This premise would be especially indicated if a portion of the watershed of any one drainage system were left intact. Insect drift from the untreated portion would colonize the lower

Table 2.—Number of genera of stream insects (excluding Odonata) found 1 week after a DDT aerial woodland spray, based on 10 foot-square samples per stream.

	STREAM				
	Hamden I	Bethany I	Bethany II	Bethany III	Cheshire (Check)
Survived[a]	14	12	16	13	19
Eliminated[b]	6	9	6	6	4
Survived[c]	1	0	6	1	3
Possibly eliminated[d]	10	12	6	14	8
Minimum loss (%)	28.6	42.8	21.4	30.0	15.4
Maximum loss (%)	51.6	63.6	35.3	58.8	35.3

[a] Found in both pretreatment and posttreatment counts.
[b] Found only in pretreatment counts in numbers of five or more.
[c] Found only in posttreatment counts in numbers less than five.
[d] Found only in pretreatment counts in numbers less than five.

reaches of the main channel of the treated parts. The following year gravid females would deposit eggs in the upper reaches of the treated areas. If a whole watershed were treated, it would be presumed that recolonization would take considerably longer.

In any stream natural catastrophes cause considerable loss in the insect fauna. Various authors have reported such causes as floods and anchor ice. These losses, however, would tend to be somewhat less selective than those from DDT poisoning. One application of an aerial DDT spray for gypsy moth control, as now practised in Connecticut, does not appear to be cause for undue alarm as to its effect on insects of streams.

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Sampling Alfalfa Seed for Clover Seed Chalcid Damage[1]

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ABSTRACT

A method is presented for rapidly estimating the per cent of harvested alfalfa seeds damaged by the clover seed chalcid, *Bruchophagus gibbus* (Boh.). The method utilizes a technique of cementing a sample of seeds on paper to permit rapid microscopic examination.

Three sampling experiments were conducted to determine the variance components associated with individual steps in the process of determining the level of chalcid infestation in a particular treatment. An optimum sampling scheme was developed from these components and from the cost of conducting the experiments. Two field samples per treatment were each subsampled twice. Two separate estimates of infestation were made on each subsample. This scheme permitted the best estimation of a treatment mean within 15% of the true mean, 95% of the time.

The clover seed chalcid, *Bruchophagus gibbus* (Boh.), currently poses a serious threat to the production of alfalfa seed in western United States. Several years ago, losses from this pest had a contributing effect in shifting the alfalfa seed production center of California from the Imperial Valley to the San Joaquin. Losses are now causing alarm in the San Joaquin Valley. In Fresno County, alone, during 1959, this insect caused an estimated $1,500,000 loss (Sarquis 1959) which represents about 15% of the total seed crop (Bacon *et al.* 1959). Some growers in the San Joaquin Valley have reported losses as high as 60% and other growers have claimed that their losses become more severe each year.

The bionomics of the clover seed chalcid have been reported by Urbahns (1920), Sorenson (1934), and, more recently, by Neunzig & Gyrisco (1959).

Researches directed toward controlling this pest are currently underway at several of the western experiment stations and a few private seed firms. Unfortunately, no effective control measures have been achieved to date, either through the use of chemicals or resistant varieties (Bacon *et al.* 1959). One of the difficulties hampering research efforts has been a lack of efficient means for evaluating a particular treatment, as well as a lack of informa-

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tion concerning the variation associated with the individual steps when sampling to determine clover seed chalcid infestations.

The problems of evaluating a treatment for seed chalcid control may perhaps best be indicated by noting the steps involved in sampling a plot to determine chalcid infestation. One or more field samples, consisting of dried pods, is drawn from the plot and taken to the laboratory and threshed. The threshed seeds are usually cleaned and a subsample is then taken. This subsample may be examined for chalcid infestation in its entirety or it may in turn be subsampled. Determining the actual per cent infestation is done by checking microscopically the individual seeds of the last mentioned subsample for soundness.

In each of these steps there is an unknown amount of variance which contributes to the inaccuracy with which an experimenter attempts to estimate chalcid infestation in a particular treatment. For example, there are errors in drawing the field samples from the plots, influenced by such items as the size of the sample, the age of the racemes selected for harvest and the position of these racemes on the plant. Both threshing and cleaning introduce errors, for, as the infested seeds are hollow they may be broken upon threshing and lost; as they are lighter, they may be separated by the blower from the sound seed upon cleaning. Errors are also present in subsampling the threshed seed as well as in determining the final per cent infestation.

The number of treatments that can be simultaneously compared is often limited by the effort necessary for making a final determination, *i.e.*, individually examining several hundred seeds.

The objectives of this study were 1) to develop an efficient method of determining the per cent infestation of seed chalcid in a lot of harvested seed, 2) to examine the components of variance associated with the individual steps in making such a determination, and 3) to devise an optimum sampling scheme so that the mean per cent infestation of a treatment may be estimated within 15% of the true mean, 95% of the time.

METHODS.—*Determining Per Cent Infestation.*—Five fields in Fresno County were sampled in August, 1959. Each sample was a composite of racemes taken from several locations within a field, and was approximately 5 quarts in volume. Three hundred milliliters of pods were selected at random from each lot of seed and threshed by rubbing between two rubber-lined boards. The threshed seeds were cleaned with the aid of a Clipper Seed Cleaner, equipped with a blower and a No. 14 upper and a No. 21 lower screen. The cleaned seeds were then subsampled three times with a small scoop which held about 400 seeds. These seeds were examined under a stereoscopic microscope to determine the per cent chalcid infestation. This step involving examination of seeds microscopically to obtain the per cent infestation will hereafter be called a "determination." The method is in common use, and is referred to here as the "scoop" method.

A new method, referred to as the "strip" method was developed as follows: two subsamples, each equivalent to 80 ml. of pods were drawn at random from each of the same five lots of seed mentioned above. These subsamples were threshed using a hand-operated, rubber-lined, rotary thresher. The threshed seeds were partly cleaned by hand screening through a No. 14 upper and a No. 22 lower screen. The fine chaff produced in threshing was not removed with an air blast, as was done when the Clipper Seed Cleaner was used. Three strips of rubber cement, $\frac{1}{4}''$ by 10″ were then applied with the aid of a template to an $8\frac{1}{2}''$ by 11″ sheet of paper. These strips were arranged in the shape of an "N." While the cement was still wet, the partly cleaned seeds from the 80 ml. subsamples were poured through a No. 13 screen and allowed to fall from a height no greater than 2 inches onto the sheet of paper holding the strips of cement. The screen was shaken and the seeds poured in such a manner that they uniformly covered the entire sheet of paper. After the cement had set, the excess seeds were gently poured off, leaving three strips of affixed seeds. Thus, every lot yielded six strips, three from each of its two subsamples. A fair amount of chaff adhered to the strips of cement. However, the sound and infested seeds were distinguished easily with a stereoscopic microscope.

Arranging the seeds in long narrow strips had several advantages. As the strips were not wider than the microscope's field of vision, counting time was materially reduced. A mechanical hand tally was used and counted seeds were marked with a pencil as a strip was pushed from left to right. Also, the seeds remained firmly in place which expedited any necessary dissections. Lastly, the paper with the cemented seeds could be filed in a loose-leaf notebook for future reference.

The costs of determining the per cent infestation within a single sample by both the "scoop" and "strip" methods were calculated on the basis of the time required to complete the sample. The pertinent variance components were then computed after an analysis of variance was performed on the mean infestations of the five seed lots. Fisher (1949) has defined information (I) as the reciprocal of the error variance of a mean. The efficiency of an experiment, then, will increase as the Cost/I ratio decreases. From this relationship, the efficiency of the two methods was estimated by comparing the Cost/I ratios of each.

Determining Variance Components.—Three sampling experiments were conducted in order to estimate the variance components associated with each step employed for determining the per cent chalcid infestation of a plot. The first was superimposed on a fertilization trial consisting of 13 treatments conducted by the Department of Agronomy, University of California, Davis. Four field samples were drawn from four of the 13 treatments. Only the center two rows of each plot, which measured six rows by 100 feet, were sampled. Each field sample was equivalent to 80 ml. of dried pods; no subsamples were taken, *i.e.*, the entire field samples were threshed. Three "determinations" were made on each field sample. The method of threshing and making determinations in all three sampling studies utilized the strip method mentioned above.

The second sampling experiment was superimposed on a row-spacing trial, also conducted by the Department of Agronomy. Three 250-ml. field samples were drawn from each of three plots, two rows by 100 feet long. Each field sample was subsampled twice; the subsamples were 80 ml. in volume, and three determinations were made on each subsample.

Table 1.—Treatments in parent trials sampled and mean per cent damage of each. West Side Field Station, Fresno County, 1959.

Parent Trial					
Fertilization		Row Spacing	Damage (%)	Resistance Trial	
Treatment	Damage (%)			Variety	Damage (%)
Check	12.1	24″	17.5	Ariz. Syn. M-56-11	30.4[a]
Nitrogen: 40 lb./A.	11.8	48″	25.2[a]	Ariz. Syn. Bam 17	48.7
Gypsum: 3 tons/A.	11.6	72″	20.8	Moapa	41.9
N:P:K; 40:96:85 lb./A., + Gypsum: 3 tons/A.	10.8			Africa	42.5

[a] Significantly different from the other treatments within the same trial at the 0.01 probability level.

A third sampling experiment was superimposed on a trial to evaluate 14 varieties of alfalfa for resistance to the seed chalcid. The varieties were seeded in single rows, 25 feet long. Two field samples, each 375 ml., were drawn at random from four of the varieties. These were subsampled twice (80 ml.) and again three determinations were made on each subsample.

Each experiment was replicated four times, and the results of the determinations were analyzed separately, after which the components of variance were computed in the usual manner. The significance and methods of handling these variance components are discussed later. Costs associated with the individual sampling steps were derived from the times required to complete that step.

A restriction was placed on the random selection of racemes from the plots. In order to reduce the expected variability of infestation levels between successive seed sets, only racemes of the same age were harvested by selecting those with greenish rachises rather than completely brown ones. All three sampling studies were conducted at the West Side Field Station in Fresno County. The individual treatments sampled from the parent trials are shown in table 1.

RESULTS AND DISCUSSION.—*Determining Per Cent Infestation.*—The percentages of infestation in five lots of seed, as determined by the scoop and strip methods are shown in table 2 and the analyses of variance of these data are shown in table 3.

By the new method, 2 hours, or $3.00 of an assistant's labor, were necessary to subsample each lot of seed twice, thresh the subsamples, prepare cement strips and examine the seeds for soundness. Thus, using the strip method, $0.60 was required to determine the per cent infestation of a single seed lot. By the scoop method, 8 hours and 45 minutes were needed to thresh all the samples, clean them, and make three determinations on each. This was equivalent to $2.63 per seed lot.

Looking at table 3, the important figure is that which estimates the variation between individual scoops or strips upon which the determinations were made, symbolized as σ_s^2. The goal of a sampling scheme is to obtain maximum information per unit cost, or to minimize the quantity Cost/Information.

As previously stated, information is defined as the reciprocal of the error variance of a mean, $I = 1/\sigma_{\bar{x}}^2$, or if the mean is a mean difference between two treatments, then

$$I = \frac{1}{2\sigma_{\bar{x}}^2} = \frac{1}{\sigma_{\bar{d}}^2}.$$

But $2\sigma_{\bar{x}}^2 = 2\sigma_s^2/n$, where n is the total number of observations (determinations) in a single mean. Using σ_s^2 from table 3,

$$\sigma_{\bar{d}}^2 = \frac{2 \times 6.3747}{6} = 2.125,$$

for the strip method, and for the scoop method,

$$\sigma_{\bar{d}}^2 = \frac{2 \times 2.2840}{3} = 1.522.$$

As

$$\frac{\text{Cost}}{I} = \frac{\text{Cost}}{\frac{1}{\sigma_{\bar{d}}^2}} = (\text{Cost})(\sigma_{\bar{d}}^2),$$

then the ratio

$$\frac{(\text{Cost})(\sigma_{\bar{d}}^2) \cdots \text{scoop method}}{(\text{Cost})(\sigma_{\bar{d}}^2) \cdots \text{strip method}}$$

Table 2.—Mean per cent infestation of the clover seed chalcid in five lots of seed determined by two different methods.

	Strip Method		Scoop Method	
Lot Number	Per Cent Infested	Total Seeds Examined	Per Cent Infested	Total Seeds Examined
1	17.6	1,951	15.0	1,170
2	20.4	1,823	18.1	1,326
3	10.0	2,191	9.4	1,594
4	41.0	1,321	37.2	1,188
5	7.0	2,113	6.7	1,382

Table 3.—Analysis of variance of clover seed chalcid infestations in five lots of seed, determined by two methods.

Source of Variance	D.F.	M.S.	M.S. Estimates
Strip Method			
Among lots	4	544.9455	
Among subsamples within lots	5	6.3747	σ_s^2
Among determinations within subsamples	20	1.2428	
Scoop Method			
Among lots	4	239.2675	
Among determinations within lots	10	2.2840	σ_s^2

is estimated as

$$\frac{(2.63)(1.522)}{(0.60)(2.125)} = 3.14.$$

This figure indicated the relative efficiency of the two methods. Thus, it can be concluded that the strip method yielded three times more information than the scoop method for a given cost.

The presence of cracked seeds in the final determination poses a difficult question. If an accurate estimate of per cent chalcid infestation is desired, should the experimenter consider a fragment of cracked seed as a whole seed, or should perhaps two or even three fragments be made equivalent to a single seed? After examining the chaff produced when threshing and cleaning seed by the strip method, apparently more accurate estimates were obtained when single parts of cracked seeds were classified as whole seeds. According to the procedure adopted for the scoop method, two seed fragments were considered to be one whole seed. This may have contributed to the lower percentages observed when samples were processed by the scoop method.

Determination of Variance Components.—Results of the analyses of variance and the variance components for the three sampling studies are shown in table 4. The most variable component of variance between the three experiments was error. However, as the three studies were superimposed on different trials and different sampling schemes were used, it is not possible to directly compare these error components. The magnitude of the variation between these components was not large enough to be significant, according to Bartlett's test (*in* Snedecor 1950) for homogeneity of error. Thus, the remaining variance components can likely be compared. Consideration of those components for field samples pertaining to the fertilization and row spacing trials (table 4) leads to the inference that there is no difference between field samples of 80 or 250 ml. in size. Field samples of 375 ml. (resistance trial), however, appear to be less variable.

The significant difference between field samples within all three experiments (table 4) was not anticipated. No studies were performed to ascertain how large field samples must be before they no longer differed significantly.

For some obscure reason a significant difference occurred between subsamples of the row spacing trial, although the contribution to variance by these subsamples was small (see table 4). Table 4 also illustrates that determinations varied about half as much as field samples in both the fertilization and row spacing trial. In the resistance trial, however, the reverse was true, but it should be recalled that the mean infestation of the resistance trial was considerably greater than that of the other two experiments (table 2) which would tend to make these determinations more variable.

Table 4.—Analysis of variance of three sampling experiments superimposed on different alfalfa trials.

Experiment	Source of Variation	N	D.F.	M.S.	V.C.
Fertilization	Treatments	4	3	17.7341	
	Blocks	4	3	10.1721	
	Error		9	143.7279	10.691
	Field samples	4	48	15.4362**	4.322
	Determinations	3	128	2.4700	2.470
Row spacing	Widths	3	2	529.6634**	
	Blocks	4	3	106.7697	
	Error		6	47.7581	0.767
	Field samples	2	24	33.9476**	4.437
	Subsamples	2	36	7.3246**	1.711
	Determinations	3	144	2.1910	2.191
Resistance	Varieties	4	3	1022.2576**	
	Blocks	4	3	198.8516	
	Error		9	191.9833	14.580
	Field samples	2	16	17.2027**	1.918
	Subsamples	2	32	5.3261	—[a]
	Determinations	3	128	5.7060	5.706

[a] This component was actually negative and hence was considered to be zero.

Table 5.—Pooled analysis and average variance components of three sampling experiments to determine the variation associated with sampling for clover seed chalcid infestations.

Source of Variation	N	D.F.	M.S.	M.S. Estimates[a]	V.C.
Error	—	24	137.8312	$\sigma_d^2+d\sigma_s^2+ds\sigma_f^2+dsf\sigma_r^2$	6.5014
Field samples	3	88	20.8059	$\sigma_d^2+d\sigma_s^2+ds\sigma_f^2$	2.4036
Subsamples	2	68	6.3841	$\sigma_d^2+d\sigma_s^2$	0.9930
Determinations	3	400	3.4050	σ_d^2	3.4050

[a] σ_d^2 is the variation between determinations.
σ_s^2 is the variation between like subsamples and in addition to that due to determinations.
σ_f^2 is the variation between like field samples and in addition to that due to determinations and subsamples.
σ_r^2 is the variation between plots receiving like treatments and in addition to that due to determinations, subsamples and field samples.

Since Bartlett's test for homogeneity of error variance indicated no heteroscedasticity, it seemed logical to pool the three analyses in order to arrive at average components of variance. Insofar as the experimental plots were of different sizes and involved different sampling schemes, the appropriate method for obtaining an estimate of the average value of each component is uncertain. In this case, the average values were obtained by pooling the mean squares weighted by the degrees of freedom. These values are shown together with the total degrees of freedom in table 5.

The costs of collecting the samples in the three experiments were not proportional to the size of the samples, for the growing conditions of the plants influenced to a large extent the ease with which a field sample could be drawn. Thus, in determining the optimum sampling scheme (see below) the costs for the row spacing experiment were used, for here it was felt that the cost of procuring a field sample was most representative. These costs are reported in table 6.

Determination of the Optimum Sampling Scheme.—Schultz (1955), in an admirable discussion covering optimum allocation of experimental material, has presented "A fairly realistic expression of cost . . . of a single treatment mean . . . ." for an experiment having r

Table 6.—Average costs of conducting the row-spacing experiment.

Step in Sampling Scheme	Cost of First Unit C	Cost of Additional Units C'
Replications	$25.00[a]	$2.00[a]
Field samples	.30	.25
Subsamples	.10	.05
Determinations	.05	.05

[a] Estimated.

Table 7.—Replications required to attain a given level of accuracy.[a]

Level of Accuracy Expressed as Per Cent of True Difference	Replications Required
± 5%	23
± 8%	9
±10%	4
±15%	3
±20%	2

[a] Student's t taken at 0.05 probability.

replications, f field samples and d determinations per field sample. An extension of Schultz's cost function suitable to the row-spacing experiment outlined in table 1 is:

$$C_{\bar{x}} = C_r(r-1)C_r' + r[C_f + (f-1)C_f'] + rf[C_s + (s-1)C_s'] + rfs[C_d + (d-1)C_d'] \quad [1]$$

where $C_{\bar{x}}$ is the cost of a single treatment mean and r, f, s, and d indicate the replications, field samples, subsamples and determinations, respectively or, if used as coefficients, the numbers of such units. C and C' represent the cost of the first and additional units after the first, respectively. This function allows for the common occurrence that overhead cost at any level may make the first unit cost more than additional ones. The cost of the mean difference between two treatments is $2C_{\bar{x}} = C_{\bar{d}}$.

Recognizing that the problem of optimum allocation is that of minimizing the quantity $(C_{\bar{d}})(\sigma_{\bar{d}}^2)$, Schultz (1955) has offered ways of determining the optimum method of sampling. For the row-spacing experiment discussed here, Schultz's formulae can be extended to read as follows:

$$d' = \sqrt{\frac{C_s' + C_d - C_d'}{C_d'} \cdot \frac{\sigma_d^2}{\sigma_s^2}} \quad [2]$$

$$s' = \sqrt{\frac{C_f' + C_s - C_s'}{C_s'} \cdot \frac{\sigma_s^2}{\sigma_f^2}} \quad [3]$$

$$f' = \sqrt{\frac{C_r' + C_f - C_f'}{C_f'} \cdot \frac{\sigma_f^2}{\sigma_r^2}} \quad [4]$$

where d', s', and f' are the optimum number of determinations per subsample, subsample per field sample, and field samples per replication.

Substituting the data from tables 5 and 6 into formulae [2], [3], and [4]:

$$d' = \sqrt{\frac{.05 + .05 - .05}{.05} \cdot \frac{3.4050}{0.9930}} = 1.18 = 2$$

$$s' = \sqrt{\frac{.25 + .10 - .05}{.05} \cdot \frac{0.9930}{2.4036}} = 1.57 = 2$$

$$f' = \sqrt{\frac{2.00 + .30 - .25}{.25} \cdot \frac{2.4036}{6.5014}} = 1.74 = 2$$

The number of replications required will, of course, depend upon the desired precision of the experiment. Schultz (1955) discusses a method of finding the required replications assuming optimum allocation of other experimental material and that the investigation will bear all costs.

A modification of the formula to find the number of replications necessary to estimate the true difference of a treatment means within a specified range at a given probability level for the row spacing experiment is:

$$r' = \frac{2t^2\left(\frac{\sigma_d^2}{d's'f'} + \frac{\sigma_s^2}{s'f'} + \frac{\sigma_f^2}{f'} + \sigma_r^2\right)}{u^2} \quad [5]$$

where r' is the required number of replications, t is any level of Student's t which suits the experimenter, and u is the observed range of treatment differences which is not to be exceeded at level t. From formula [5] the number of replications required for different levels of accuracy were computed and are reported in table 7.

Thus it may be concluded that under the conditions of the row-spacing experiment, two determinations made on each of two 80-ml. subsamples taken from each of two 250-ml. field samples, replicated three times, will permit the experimenter to estimate the per cent clover seed chalcid infestation within 15% of the true difference, 95 per cent of the time. This particular sampling scheme may be satisfactorily used by other workers only if their cost functions are similar to those reported here. The variance components found in this investigation may be useful to other investigators for planning experiments. The general method of arriving at an optimum sampling scheme is also applicable to related types of work.

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The Beet Armyworm, *Spodoptera exigua*, an Economic Pest of Citrus in California[1]

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Abstract

Larvae of the beet armyworm, *Spodoptera exigua* (Hbn.), caused substantial economic losses in approximately 500 acres of young, recently planted citrus trees and nursery stock during the summer and fall of 1957, 1958, and 1959 in most California citrus areas. The larvae may destroy each succeeding flush of growth and the terminal shoots. The attacks of the larvae have a pruning effect on the young trees, and subsequent retarded tree growth causes infested trees to develop only half as fast as noninfested trees. The new rootstock-scion combinations currently being planted grow two or more times as rapidly as former combinations; consequently, the continuous supply of young and succulent foliage provides food for optimum development of beet armyworm larvae. The facts seem to explain the recent "adaptation" of the beet armyworm, enabling it to infest citrus and cause serious economic losses. The life cycle of this insect lasts 30 to 60 days during the summer and fall, and there are four or more generations a year on citrus in California. No parasites have been taken on citrus thus far. Chemical control measures utilizing DDT and parathion provided excellent but short-term control, since reinfestation was continuous.

In the past three years (1957–1959), the beet armyworm, *Spodoptera exigua* (Hbn.),[2] has become an economic pest of young citrus plantings in several widely separated areas in California. The damage inflicted was confined primarily to expanding foliage and terminal shoots, which were damaged or destroyed in young citrus plantings and in nursery beds. This damage causes a materially retarded rate of growth in the infested plantings.

Synonymy.—The scientific name of the beet armyworm was designated *Spodoptera exigua* (Hbn.), a new combination, with the previous generic name, *Laphygma exigua* (Hbn.), becoming a new synonym (E. C. Zimmerman 1958). Zimmerman indicates that the genus *Spodoptera* Guenee has chronological preference over the genus *Laphygma* Guenee since the two apparently do not differ adequately to warrant the use of more than one genus.

Historical.—*Spodoptera exigua* was first described from North America by Harvey (1876) from adult specimens from California and Oregon. Subsequently, many collections were made in California by other workers. The earliest record of the larvae of the beet armyworm in the United States was reported by D. W. Coquillett on May 25, 1882, when a larva was taken at Anaheim, California (Chittenden 1902).

Distribution.—Records of economic infestations of the beet armyworm on citrus in California available to the author are as follows:

Tulare County—Lindsay, July and August 1957 and 1958 (young citrus trees); Lindsay, July and August 1959; Woodlake, Terra Bella, Strathmore, and Porterville, July 1959 (all on young citrus trees).

Riverside County—Riverside, August 1959, and Corona, September 1959 (in nursery of young citrus seedlings); Coachella, July 1958 and 1959, and Hemet, July 1959 (on young citrus trees).

Ventura County—Fillmore, October and November 1958 (citrus seedlings in nursery bed).

In addition, larvae have been taken from citrus or other hosts in every county in the California citrus belt. The first published account of the beet armyworm attacking citrus was apparently that described in the U. S. Department of Agriculture Cooperative Economic Insect Report (C.E.I.R.) 8(10): 180, 1958.

Biology.—The life history and habits of the beet armyworm have been described for the California area on citrus.

Egg Stage.—The eggs measure 0.5 mm. in diameter; they are spherical, with radiating longitudinal lines, and are iridescent pearlaceous to pink in color. Moths obtained from larvae reared on citrus were found to produce an average of 520 eggs per moth. Each female moth deposits several egg masses which averaged 83 eggs per mass.

Larval Stage.—Newly hatched larvae are approximately 1 mm. long, with a comparatively large blackish head and a light-green body covered with the usual complement of noctuid spines. As growth proceeds and molting occurs, the coloration varies greatly, so that there is no typical color pattern for any instar.

The full grown larvae (see fig. 1a) measure 25 to 35 mm. in length and 4 mm. in breadth. Head: 2 mm. wide, brownish, with a number of black-margined, near-white reticulations on the epicranium. Thorax: light-to-dark-brown prothoracic shield, marked by one median and two lateral pale lines; a dark spot is usually present on each side of the mesothorax; thoracic legs brown. Abdomen: spiracles white or yellowish within the black peritremes; a small pale spot dorso-posterior to each abdominal spiracle; skin smooth; general color varying from green, through soft shades of gray, to brown; dorsum, between the light, narrow, subdorsal lines, dotted and lined confusedly in green or near black and heaviest near pale dorso-mesal line; area between subdorsal lines and the broad subspiracular greenish band gray to black, strongly dotted with near-white spots; venter, pale and mottled with white; prolegs, green. Color description somewhat adapted from that of Peterson (1948), Crumb (1956), and Okumura (1959).

Wilson (1934) contends that light and dark-colored individuals have been reared from the same egg mass, though the dark larvae are more numerous in the early spring, late fall, and winter, and the lighter larvae are more numerous during the summer months. He states that this indicates a close correlation between low tem-

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[2] Lepidoptera: Noctuidae.

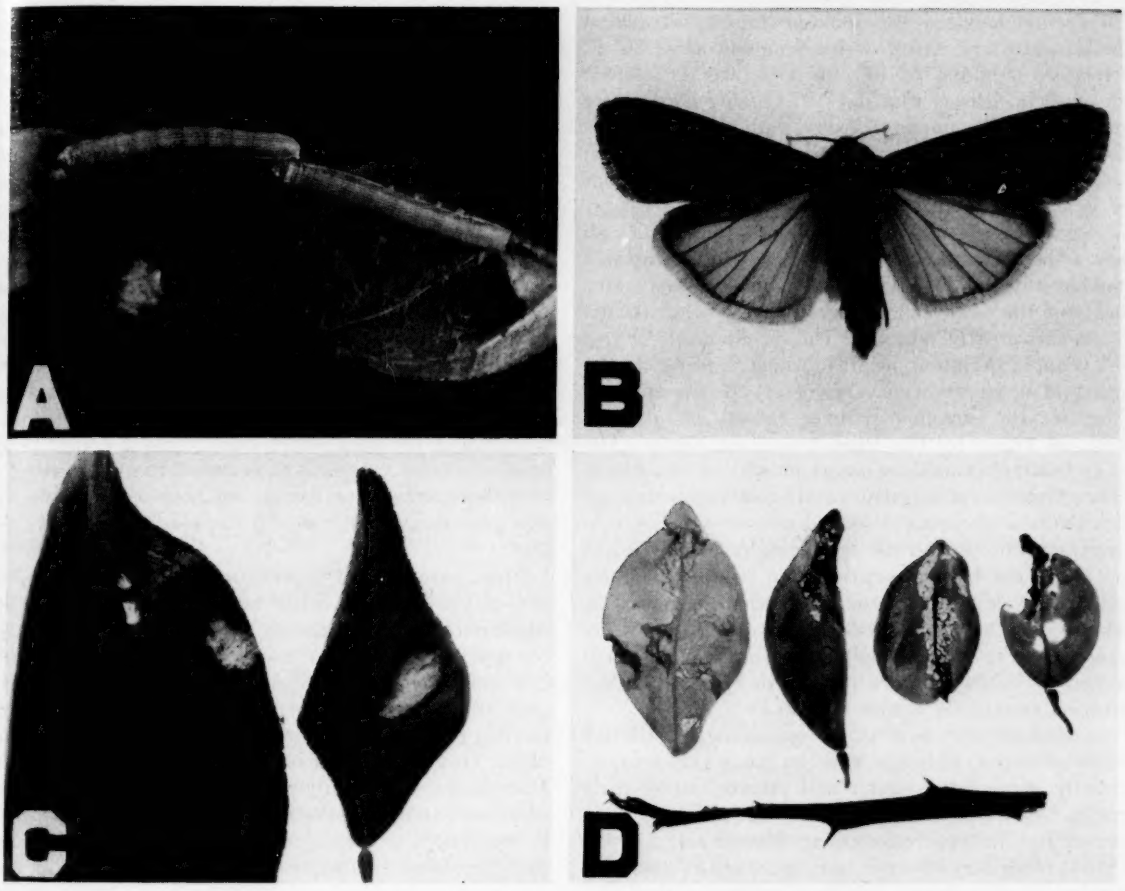

FIG. 1.—The beet armyworm, *Spodoptera exigua* (Hbn.), an economic pest of citrus in California (Lepidoptera: Noctuidae).
(a) Full-grown larvae in light-color phase, feeding on citrus leaf (length, 25 to 35 mm.);
(b) Adult moth (wingspread, 32 mm.);
(c) Typical egg masses (8 to 10 mm. long) on upper surfaces of citrus leaves; and
(d) Typical damage by beet armyworm to citrus foliage and terminal shoot.

perature and dark pigmentation and between high temperature and light pigmentation.

Pupal Stage.—The pupae are light bro[illegible]th margins of abdominal segments darker brown. L[illegible], 15 to 20 mm.

Adult Stage.—The adult moths are plain grayish to brownish in color (see fig. 1b), and are similar to other typical cutworm moths, except that they are slightly smaller. Forewings: mottled gray or brownish, with a lighter spot near the center of each wing. Hindwings: paler in color than forewings, blending to darker near the edges, around which there is a light band of cilia. Wing expanse, 25 to 30 mm. Color of abdomen is similar to that of forewings.

LIFE HISTORY.—The eggs are commonly laid in clusters on the upper surfaces of recently mature citrus leaves. They are usually in somewhat irregular rows and occasionally in more than one layer. The egg masses are covered with hairs and scales from the female moth's body, these adhere to the sticky eggs during egg deposition, giving the cluster the appearance of a small irregular tuft of cotton (see fig. 1c).

The larvae hatch by chewing a hole in the top of the egg shell, through which the caterpillar escapes. After emergence the larvae sometimes consume the egg shell. All of the larvae from one egg mass emerge at approximately the same time and frequently remain together, feeding gregariously under a few filaments of webbing on young nearby foliage, on a terminal leaf cluster, or on the surface of a single leaf. They may remain together until they are almost half grown (third instar) and are $\frac{1}{2}$ inch long. The older larvae frequently scatter and may be found on any part of the young citrus tree, but usually on tender young growth and in the proximity of the hatched egg mass.

There are a total of five instars. When the larvae are full grown they leave the tree, and the body shortens as they enter the prepupal stage prior to transforming to the pupal stage. Pupation takes place in a thin, loose cocoon under leaf trash or just under the surface of the soil near the trunks of young trees.

The adult moths emerge during the night, and mating takes place soon after the female reaches the surface of the ground. There is a preoviposition period of approximately 2 days; oviposition continues for 3 to 7 days and is followed by a short postoviposition period of about 1 day.

The average length of the different stages when reared in the laboratory on citrus, with a temperature of 80° F. and relative humidity of 40% to 55% was as follows: eggs, 4.4 days; larvae, 21.3 days; (including 1.7 days for prepupal interval); pupae, 8.7 days; adults, 8 days (females, 7 days, with preoviposition period of 2 days; males, 9 days). Thus the average length of life cycle under these laboratory conditions was 36.4 days.

SEASONAL HISTORY.—In the California citrus belt all stages of the beet armyworm may be present in any season and at any one time. The insect is more or less active throughout the year, but during cooler weather its development is greatly retarded. The development of this insect is rapid beginning in May, which is probably the month that first-generation larvae start their population buildup for the economic damage caused in July and August on citrus by the third-generation larvae. The first- and second-generation larval population levels have not been high enough on citrus to cause economic damage so far. Because of chemical control measures during subsequent generations and the hardening off of the young citrus trees, the later generations are reduced to lower population levels and begin to be somewhat uneven. This, together with the lower mean temperatures, slows development so that only a small portion of the last generation and probably a larger portion of the next-to-last generation go into the winter months.

It is assumed that most of the continuing population survives as pupae, although some larvae in this area undoubtedly survive the winter and pupate during early spring to begin the first generation of the following year. There are four full generations of armyworm and it is possible that there may be six or more generations under optimum weather conditions and with a plentiful food supply present on vigorously growing young citrus trees. The length of the life cycle in the field on citrus, during July and August, is approximately 30 to 45 days for the third and fourth generations.

NATURE OF DAMAGE.—The feeding of the larvae of the beet armyworm on young citrus trees is confined primarily to the tender, expanding growth. However, the tips of young twigs are destroyed in the heaviest infestations, as shown in figure 1d The young larvae cause a skeletonizing type of damage on either the upper or the lower surface of the leaf. As the larvae become larger they make holes through the leaf and often consume the entire leaf. In heavy infestations the young twigs are stripped of their leaves and the secondary leaf buds are destroyed.

The overall result of the attacks by the armyworm larvae is a pruning effect on the young citrus trees, which causes branching of the shoots instead of elongating into a desirable straight scaffold. In addition, the total growth of the tree is substantially retarded, so that the damaged trees increase in size only half as much as the undamaged trees.

Population levels of beet armyworm larvae were as high in noncultivated groves as in cultivated groves. This is probably because a larger number of pupae are destroyed in the cultivated groves. One grove in Tulare County had some pigweed (*Amaranthus*) growing in the grove. These weeds were infested with larvae but not as heavily as the young citrus trees. This would indicate that the tender citrus growth is preferred to the pigweed.

The final result of a heavy infestation of the beet armyworm on young citrus trees is identical with that caused by the larvae of the anise swallowtail butterfly, *Papilio zelicaon* Lucas (Atkins 1960a), and the cabbage looper, *Trichoplusia ni* (Hbn.), (Atkins 1960b), which are also serious economic pests of young citrus trees in California.

The population levels of these worms were excessively high as each small tree (foliage area approximately 3 feet in diameter) had an average of 27 caterpillars per tree in a 50-acre grove. Utilizing our traditional counting method (per-hour-search basis) to determine the population level for "orangeworms," the population levels in this grove averaged 200 to 350 beet armyworm larvae per hour (for explanation of this method, see Atkins *et al.* 1957). Population levels in other infested groves revealed up to 230 larvae on a per-hour-search basis. Groves considered to have economic populations revealed the presence of 50 to 60 beet armyworm larvae per-hour-search basis; this was an average of 2 to 6 of these insects per small citrus tree.

From careful field observations and from consideration of the extensive list of host plants it is known that *Spodoptera exigua* oviposits somewhat indiscriminately on any species of plant available. Its ability to become of economic importance on a particular plant species is probably caused by the suitability of that particular plant species as food for larval development; the severity of the economic attack is owing to the fact that such a plant species is a highly suitable host for the completion of more than one generation of the beet armyworm. This is certainly the reason in the case of these vigorously growing young citrus trees.

Almost all of the recently planted citrus trees are composed of the following scions: nucellar Frost navel orange; nucellar Campbell, Frost, Olinda, or Cutter Valencia orange. These are grafted onto rootstocks of nucellar Troyer citrange or nucellar trifoliate orange. These rootstock-scion combinations grow two or more times as rapidly as former combinations. Consequently, a continuous supply of young and succulent foliage for optimum development of beet armyworm larvae is provided. It is believed that the above facts explain the recent "adaptation" of the beet armyworm, thus enabling it to infest citrus and cause serious economic losses.

NATURAL CONTROL.—Birds of various species have been seen in Tulare County collecting beet armyworm larvae and eating them in the grove or flying away with them. Although Campbell & Duran (1929) found many parasites in their work, no parasites have been observed so far in the present studies. This could easily be true since young citrus plantings are frequently treated with pesticides for various mite and insect pests throughout the season. Most of the larvae brought into the laboratory to be reared in petri dishes died as a result of a polyhedrosis virus infection when in the fourth or fifth instars. However, no diseased larvae were found in the field.

CHEMICAL CONTROL.—Commercial pest control operators used DDT, parathion, malathion, and, in one instance, Sevin® (1-naphthyl methylcarbamate), in sprays during the year 1958–59 to control the larvae of the beet armyworm on nonbearing young citrus plantings and in citrus nursery beds. DDT, 50% (2 pounds of wet-

table powder per 100 gallons) gave excellent control and the longest residual protection; 25% parathion (2 pounds of WP per 100 gallons) gave excellent control but short residual protection; 25% malathion (4 pounds of WP per 100 gallons) gave poor control and no apparent residual protection; 50% Sevin (4 pounds of WP per 100 gallons) gave only fair control but exhibited no residual protection in this single trial. No difficulty was experienced in controlling the larvae with either DDT or parathion. Because the trees grow so profusely the importance of a residual insecticide is minimized. Field observations have shown that a chemical treatment is required every 2 to 3 weeks to provide adequate protection because reinfestation is continuous.

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Observations on the Biology of *Psorophora cyanescens*[1]

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ABSTRACT

The bionomics of *Psorophora cyanescens* (Coq.) have been studied over a period of years in the Tennessee Valley. The adult females attack man readily day or night when their habitats are invaded. However, the principal period of dispersal and feeding is in the evening crepuscular period.

Larvae of *P. cyanescens* have been most commonly collected in temporarily flooded, open grassland depressions and ditches. Overwintered eggs in an experimental basin did not hatch when flooded on April 2 to 4 with river water at 59° F. temperature and when the soil temperature remained below 76° F. Hatching occurred in a subsequent flooding on April 29 to 30 when the soil temperature reached 89° F. and the river water 70° F. The eggs of *Aedes vexans* hatched in both floodings.

Eggs of *P. cyanescens* less than 1 month old collected from females in the laboratory hatched in 1:400 dilutions of canned cream style corn, while eggs placed in strained pond water or in tap water did not hatch.

The bionomics of *Psorophora cyanescens* (Coq.) have been studied for several years in the Tennessee Valley. The females are widely known for their persistent attacks on man and livestock. The first adults appear about a week after heavy midsummer rains, and a single brood may persist for 2 or 3 weeks thereafter. While the greatest numbers in the Tennessee Valley have been observed during the hot months of July, August, and September, records indicate their presence from April 25 to October 15.

ADULT BIONOMICS.—Adults of *P. cyanescens* have been observed to rest on vegetation near their breeding area. The females are persistent and vicious biters and are rather difficult to dislodge once they have started to take a blood meal. The tenacity of this species was evidenced when females with as few as two legs were observed to land on a collector's arm, feed, and fly off. (The loss of legs may have been caused by contact with DDT on cattle in the pasture.) They feed anytime during the day or night but are most active at about dusk. The adults disperse readily at night to lighted areas. An extensive outward movement from a breeding area in a pastured bottom near Florence, Alabama, was observed in the evening crepuscular period the second day after emergence. This movement, as measured by biting observations in open situations, was mainly near the ground. Numbers biting human collectors in the 7:00 to 8:00 p.m. interval on a steel tower in the open bottom pasture about $\frac{1}{4}$ mile from their breeding site showed 1 at 60-foot level and 0 at 119-foot level, while 334 were taken at ground level. The same evening a collection from a light trap situated on a wooded bluff about $\frac{3}{8}$ mile from their breeding area yielded 24 *P. cyanescens.*

In a Tennessee River bottom hardwood forest near Sugar Tree, Tennessee, a slight diurnal biting activity of *P. cyanescens* was detected in a tree canopy at 30- and 50-foot levels during afternoon hours. Simultaneous collections at ground level in the forest were negative, but along open shoreline adults were collected in a parked

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car. Experience in Illinois has indicated that females fed primarily in the open during a daytime observation and were reluctant to enter the forest (Ross 1947). Paired collections have been made during afternoons in open pasture and willow swales and greater numbers fed in the open than in the willow swale. It appears that *P. cyanescens* prefer to stay and feed in open situations near the ground, but when they enter forested areas they may feed at higher elevations in the canopy.

Adult females of *P. cyanescens* have been collected as far as 2 miles from their probable breeding areas. Although the peak period of flight and feeding is just before and after dark, females are easily provoked to feed during the day when their resting places are invaded. At Muscle Shoals City, Alabama, heavy rains about September 1, 1950, produced a brood of *P. cyanescens* from depressions in vacant lots. On September 7 at 2:00 p.m., a sedan car was driven across one of the lots and then parked about 40 feet away. The collector remained inside with the windows open. During the next 15 minutes, 319 *P. cyanescens* were collected. Another "parked car collection" on September 13 at the same time and place produced 392 females. A final observation was made on September 26, and only one entered the parked car in the 15-minute collecting period. These observations suggest that adult activity from a single brood may not last longer than 3 weeks. In Arkansas, none were found at an isolated breeding site 17 days after emergence, although several were still active after 15 days (Schwardt 1939).

SEASONAL DEVELOPMENT IN THE LARVAL HABITAT.—Larval habitats of *P. cyanescens* in the Tennessee Valley are usually open grassy depressions and ditches which are flooded temporarily or intermittently (Snow & Pickard 1956). At one rocky area in East Tennessee, an early brood of *P. cyanescens* larvae was found in pothole basins. These basins, connected by underground passages with Norris Reservoir, are usually flooded in May or June by higher water levels. Later broods do not have an opportunity to develop because of a regular seasonal recession of the reservoir.

A very productive habitat for *P. cyanescens* was a series of small depressions in a pastured bottom (McFarland) along the right bank of the Tennessee River near Florence, Alabama. The depressions are situated above top summer pool level for Pickwick Reservoir (elevation 414.0 feet). One of these depressions, designated as experimental pond "A," has been made to produce mosquitoes for ecological studies by intermittent artificial flooding. These studies have been made over a period of years to determine mosquito selectivity of oviposition sites, species composition, density, and seasonal distribution. This experimental elongate basin, 0.6 acre in size, has a bottom elevation of 419.2 feet and an overflow drain at elevation 420.2 feet. Elevations were staked in five transects across the basin at 0.2-foot intervals. Water was pumped from the Tennessee River periodically over a period of years to flood the basin to top elevation (420.2 feet).

Survey of Oviposition Sites.—A survey of oviposition sites by soil sampling was carried out prior to each of the five artificial floodings of the experimental basin. During the season five 6-inch square samples were cut at each selected site with due respect for similar plant cover.

Table 1.—Floodwater mosquito eggs associated with two ecological situations in the fluctuation zone of an intermittently flooded basin at Florence, Alabama, 1957.

Species	Egg Density[a]					
	Apr. 29	May 14	July 10	Aug. 9	Nov. 7	Total
Grass under Osage-Orange and Ash						
Aedes vexans	416	590	91	62	466	1,625
Psorophora confinnis	114	73	87	43	83	400
P. cyanescens	625	316	16	1	114	1,072
P. discolor	85	24	12	12	96	229
Grass (no canopy)						
Aedes vexans	518	8	80	36	364	1,006
Psorophora confinnis	19	21	54	37	48	179
P. cyanescens	60	5	1	12	15	93
P. discolor	9	0	2	2	3	16

[a] Numbers based on individual 6-inch square soil samples 1-inch thick.

These surveys were used to determine the potential productivity before the basin was artificially flooded (table 1).

In general, the Horsfall survey method was used to determine the oviposition sites (Horsfall 1956a). However, instead of using the recommended trowel to cut the soil samples, a new, heavier, sharper tool was made that is more efficient in cutting through roots of grasses, sedges, et cetera. A small spade fashioned from a flattened posthole digger blade approximately 6 inches wide and provided with a 2-foot aluminum (tube) handle was used for cutting and removing the samples from the soil. The Illinois mosquito egg separator was modified slightly in that the rotary cylinder of screens and water tank were inclined at an angle of about 45 degrees and the upper end was left open to provide greater accessibility and ease of operation.

Transect soil sampling on March 25, 1957, showed that *P. cyanescens* eggs were established in the lower portion of the basin between contours 419.2 feet and 419.6 feet along with *Psorophora confinnis* (L. & A.), *P. discolor* (Coq.), and *Aedes vexans* (Meig.); however, only eggs of *A. vexans* were abundant near and slightly above elevation 420.0 feet.

For the most part, subsequent sampling was undertaken in two ecological situations occurring between elevations 419.2 to 419.4 feet (table 1). One shaded situation contained grass and sedge at the base of ash and osage orange saplings, while the other one was composed of grass and sedge with no tree cover.

Observations on seasonal hatching and change in species composition were made in connection with five artificial floodings of the experimental basin. Dates with respective maximum elevations were: April 4 (420.0 feet); April 30 (420.0 feet); May 15 (420.0 feet); July 18 (419.7 feet); and August 31 (420.0 feet). Heavy rains on July 2 caused a natural rise of water levels to elevation 419.9 feet. A gradual increase in temperature over a period of time in the early part of the growing season appeared to be necessary for hatching the overwintering eggs of *P. cyanescens*. With the first flooding on April 2 to 4, eggs of *P. cyanescens* failed to hatch although extensive numbers of *A. vexans* eggs hatched and the larvae matured. Soil temperatures in midafternoon prior to flooding were 68° F. The river water used in the flooding was

59° F. By April 24 the pool was dry. A second flooding on April 29 to 30 resulted in a widespread hatch of *P. cyanescens* and another brood of *A. vexans*. Soil temperature at noon just before flooding was 89° F. River water used in the flooding was 70° F. The inability of *P. cyanescens* to hatch in the early season while the temperatures are low confirms similar observations made the previous year.

The soil was sampled for eggs on May 14, 1957, prior to flooding. A considerable number of eggs were recovered which probably indicate an incomplete hatch of *P. cyanescens* from the April flooding. Since it is unlikely that females resulting from the April 29 to 30 inundation would have had time to oviposit and for the eggs to have reached full embryonic development in this intervening period, it appears that the large number of *P. cyanescens* eggs which hatched following the May flooding had overwintered. On the other hand, the rise in numbers of *A. vexans* eggs in the May 14 sample was attributed to deposition by females after the April 2 to 4 flooding.

In the fall few *P. cyanescens* eggs were found (table 1). This decline may have been owing to repeated or excessive floodings of the experimental pool. In grassland habitats of North Alabama where *P. cyanescens* is most prolific and often the only species encountered, standing water is usually present less than a week and then only once or twice during the summer. In regard to the higher number of *A. vexans*, the flooding of August 31 reached elevation 420.0 feet and undoubtedly favored this species which shows a wider range of oviposition sites than *P. cyanescens*. It should be noted that the greater numbers of *P. cyanescens* eggs at the beginning of the season (April 29) were in grass and sedge at the base of isolated sapling trees located below elevation 419.4 feet. The selection of oviposition sites in intermittently flooded, shaded, bottom situations is characteristic of other species in the subgenus *Janthosoma*, *i.e.*, *Psorophora ferox* (Humb.), *P. horrida* (D. & K.), and *P. varipes* (Coq.).

In Arkansas successive seasonal hatching of broods of *P. cyanescens* has been attributed to the fact that all habitats are not simultaneously flooded. On the basis of laboratory evidence, it was claimed that hatching of eggs never occurs during the same year the eggs are deposited even though the embryos appeared to be fully developed 5 days after egg deposition (Schwardt 1939).

Viability of Eggs.—In the laboratory at Wilson Dam, Alabama, it has been demonstrated that eggs of *P. cyanescens* can be artificially stimulated to hatch 24 days after deposition with subsequent development to the adult stage. Females captured during the last week in July oviposited in the laboratory on August 3 and 4. These eggs were maintained at an average temperature of 85° F. On August 27 to 28, attempts were made to stimulate the eggs to hatch by placing them in a canned cream style corn solution which caused a decrease in the dissolved oxygen content of the water. A freshly prepared broth of corn and tap water at a dilution of 1:400 was used (Horsfall 1956b). Pond water and tap water were also used. The pond water was from a recently flooded (5 days) habitat of *P. cyanescens* and was strained through a 100-mesh screen prior to use. In each test 200 cc. of each test solution was set up in 275 cc. pyrex crystallizing dishes. Bits of cellucotton on which the eggs had previously been laid (August 3 and 4) were submerged in the solutions. Groups of 14 to 18 eggs were used in each test. In a group of 18 eggs added to the corn solution on August 28, four larvae hatched within the first 4 hours. Two of the larvae matured and pupated on September 2. Emergence of the adults occurred about 24 hours later. Other tests confirmed the fact that eggs less than 1 month old would hatch when placed in the corn solution, while those in pond and tap water did not. Temperature of the medium ranged from 78° to 98° F. with an average of 86.8° F.

DISCUSSION OF RESULTS.—It is believed that *P. cyanescens* may produce a number of broods from April 25 to October 15 determined by the number and frequency of the flooding of habitats. Eggs will hatch during the season in which they are deposited provided they are flooded with a suitable medium that causes a gradual decrease in the dissolved oxygen content of the water. This was illustrated in the laboratory when 24-day old eggs were stimulated to hatch by adding a fresh corn solution. This occurs within 24 hours under natural conditions when a terrestrial pool is flooded during hot weather and decomposition of plants and bacterial action takes place. Although the pond water hatched eggs in the experimental pool within 24 hours after flooding, when the clear water was brought to the laboratory 5 days later it, alone, was obviously not a suitable medium. The midsummer decline in egg counts obtained from soil samples as shown in table 1 may be accounted for by the frequent floodings causing less suitable oviposition sites. Many of the females that had migrated in search of blood may have found other more desirable oviposition sites in the surrounding pastured bottom in which to oviposit. The large number of eggs recovered in the April soil samples were probably oviposited by females from a large single brood produced the previous summer when the basin was last flooded. Under natural conditions in the Tennessee Valley productive *P. cyanescens* habitats are usually flooded once or twice during the summer season and then only for a short duration.

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DDT Residue Studies on Peppers[1]

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Abstract

On experimental plots of peppers, residues of DDT were considerably higher on the plants than on the fruit. Doubling of the dosage did not necessarily result in doubling of residue on either plants or fruit. At 1 pound per acre the immediate average amount of DDT residue on fruit did not exceed the tolerance set (7 p p.m.); at 2 pounds per acre the tolerance was exceeded. Seven days after treatment the average amount of residue was below the tolerance except at the 2-pound rate with sticker-spreader added. Residues of DDT on fruit were reduced by processing, and residues in the canned product were below the tolerance for the raw product. Seven days after treatment residues on pepper fruit sprayed with air blast equipment at 2 pounds per acre were well below the tolerance set for DDT.

The residues of DDT were determined on pepper plants and on pepper fruit from experiments by Burbutis *et al.* (1960). In these experiments DDT was applied in emulsifiable and wettable powder formulations at rates of 1 and 2 pounds actual per acre and with and without sticker-spreader (Plyac) at the 2-pound rates. Residues were determined on whole plants immediately after spraying, on fruit at 0-, 1-, 7-, and 10-day intervals, to ascertain the rate of disappearance between harvesting periods. To observe the effect of processing on residues, samples of fruit from the various treated plots were canned in a commercial packing plant by the usual procedures, and DDT was determined on the fruit at intervals during the canning operation and in the finished product. Analyses were also made of DDT residues on both whole plants and fruit sprayed with air-blast equipment in large commercial pepper plantings, and the resulting infestations by the European corn borer (*Pyrausta nubilalis* (Hbn.)) were recorded in these same areas.

Residue Studies on Experimental Plots.—All treatments were applied in water at the rate of 100 gallons of finished spray per acre at 250 p.s.i. with an improved or "Slosser" type spray boom (Burkhardt & Ditman 1956), especially designed for spraying peppers. Plants were thoroughly wet both from above and below with this equipment. Treatments were replicated four times in randomized block experiments which were replicated three times in different pepper fields designated as A, B, C.

All chemical analyses were made at College Park, and sampling of treated pepper plants and fruit was done with a minimum of travel. On July 31, plant samples from all DDT-treated plots of experiment B were taken. Two whole plants from each plot were taken immediately after sprays were applied and dried unless otherwise noted. The whole plants were individually analyzed for DDT by the Schechter-Haller Method (Schechter *et al.* 1945). Results are given in table 1.

All fruit samples were collected on August 25. In experiment A, application of both the 7- and 10-day treatment schedules occurred on that day. By drawing samples from all plots before and after spraying, fruit at 0, 7, and 10 days from time of treatment was obtained. Fruit was also collected 24 hours after treatment from DDT-treated plots of experiment B on August 25. Field B had been sprayed the previous day. A rain of 0.55 inches fell during the 24-hour period, but sprays had dried before the rain started. Single random fruit samples of approximately 2 pounds were collected on each of two rows of each plot at each sampling providing a total of eight analyses of each treatment at each sampling period. Results are given in table 2.

Table 1.—Residues of DDT on whole pepper plants taken from experimental plots immediately after spraying.

Formulation and Pounds of DDT Actual Applied per Acre	Residues (p.p.m.)		
	Maximum	Minimum	Average
E.C., 1	45.8	29.0	35.6
E.C., 2	163.5	64.1	104.4
W.P., 1	117.5	54.6	80.9
W.P., 2	138.6	72.4	95.3
W.P., 2[a,b]	87.8	52.9	68.6
Check	6.0	2.9	4.5

[a] Plus Plyac (sticker-spreader) 4 oz. per acre.
[b] These plants were rained on following treatment before spray dried and samples could be taken.

In examining tables 1 and 2, it is evident that residues of DDT were considerably higher on foliage than on fruit, which probably results from the greater weight of fruit in relation to surface area. Doubling the amount of DDT in the spray did not necessarily result in doubling the residue.

A 0.55-inch rainfall after sprays had dried did not remove appreciable residue from pepper fruit.

On experimental plots at 1 pound per acre, average DDT deposits did not exceed official tolerance for pepper fruit immediately after spraying. Immediately following application at the 2-pound rate, tolerances for DDT (7 p.p.m.) were exceeded slightly.

DDT residues from all sprays applied with the experimental sprayer were rather persistent, but on fruit, after 7 days, residues had dropped below tolerance with both the 1- and 2-pound rates of application except when spreader-sticker was added to the spray.

There was a slight tendency for residues from emulsion sprays to persist more than the wettable powder formulation without spreader-sticker. The addition of the spreader-sticker increased the persistence of the wettable powder formulation.

Effect of Processing on DDT Residues.—Peppers of all DDT treatments from field A were taken to the packing plant on August 28, 48 hours after previous

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Table 2.—Residues of DDT on pepper fruit immediately after spraying and at 1-, 7- and 10-day intervals.

Formulation and Pounds DDT Actual Applied per Acre	Residues (p.p.m.)		
	Maximum	Minimum	Average
Immediately after spraying			
E.C., 1	11.5	4.5	6.7
E.C., 2	14.1	7.4	10.3
W.P., 1	7.0	3.5	4.7
W.P., 2	16.1	4.2	10.5
W.P., 2[a,b]	15.2	5.6	10.3
E.C., 2[a,b]	12.2	6.0	8.9
W.P., 2[a,b]	14.6	5.4	9.9
Check	—	—	—
24 hours after spraying following 0.55 in. rain			
E.C., 1	—	—	—
E.C., 2	16.3	8.2	11.9
W.P., 1	—	—	—
W.P., 2	16.7	6.0	11.0
W.P., 2[a,b]	—	—	—
E.C., 2[a,b]	17.7	6.3	11.4
W.P., 2[a,b]	13.1	7.0	9.2
Check	3.2	0.9	1.8
7 days after spraying			
E.C., 1	8.4	3.0	5.4
E.C., 2	8.5	5.4	6.9
W.P., 1	5.0	2.1	3.0
W.P., 2	9.0	3.2	5.4
Check	2.1	0.7	1.2
W.P., 2[a,b]	11.5	5.1	8.1
10 days after spraying			
E.C., 2[a,b]	7.5	3.1	5.9
W.P., 2[a,b]	11.0	2.6	6.2

[a] Plus Plyac (sticker-spreader) 4 oz. per acre.
[b] Treatments applied every 10 days—others on 7-day schedule.

treatment, and processed in the normal commercial manner. Samples for analysis were drawn before the operation began after a thorough washing of the whole fruit, after dicing and another wash, and in the can. Results are given in table 3.

Examination of table 3 shows that DDT residues were reduced but not eliminated by processing. DDT residues resulting from emulsion sprays were more persistent during processing than those of wettable powder formulations. Residues in the canned product from all treatments were below Federal tolerance.

OBSERVATIONS ON AIR BLAST SPRAYING.—For commercial treatment with the air-blast sprayer, peppers were planted in blocks of 20 rows spaced 40 inches apart. The blocks were sprayed from both sides. On July 31, a single plant was taken from each row of a single block immediately after spray had dried and the whole plants were analyzed for DDT. The rows ran east and west, and for purposes of identification, were numbered from 1 to 20, from north to south. Results of DDT determinations are given in table 4. The pattern of row by row DDT deposit would be expected to be influenced by the wind at time of treatment. Since this field was being treated on a 7-day schedule and had several treatments before sampling, and because the prevailing wind was from the southwest, highest residues were expected on rows on

Table 3.—Residues of DDT on pepper fruits from experimental plots at various stages of commercial processing.

Pounds DDT Actual Applied per Acre	Residues (p.p.m.)			
	Pre-wash	After First Wash	After Second Wash	In Can
E.C., 1	3.2	2.3	2.2	2.2
E.C., 2	4.1	2.6	3.7	4.4
W.P., 1	2.8	3.2	0.8	0.5
W.P., 2	8.4	5.3	2.2	1.0
W.P., 2[a,b]	7.7	4.0	2.5	2.0
E.C., 2[a,b]	4.2	3.2	1.9	3.0
W.P., 2[a,b]	6.9	2.1	2.3	3.4
Check	0.5	0.5	1.2	0.5

[a] Plus Plyac (sticker-spreader) 4 oz. per acre.
[b] Treatments applied every 10 days; others on 7-day schedule.

the north side of the field. This was evident in the results recorded as the average residue on the north 10 rows was higher than it was on the south 10 rows; however, there was a rather high residue level on rows next to the outside rows and on the eight rows in the center of the field. In this commercial application, DDT wettable powder was applied at 2 pounds per acre by the air-blast equipment, and it is evident from the data obtained that average DDT deposits on the plants were generally less than when 2 pounds of DDT wettable powder was applied per acre with the experimental boom sprayer. Examination of plants immediately after spraying also indicated that individual plant coverage was not as uniform as with the boom sprayer.

On August 25, samples of fruit were collected on an air-blast treated block immediately before and again immediately after spraying, and sprays had dried providing 7- and 0-day samples. Two fruit samples were collected, one from the north side and one from the south side of each row. Results of the analyses are given in table 5.

Residues of the pepper fruit were surprisingly uniform from all rows sprayed with air-blast equipment. Residues on the fruit were highest on the outside rows and the rows in the middle of the field as was found for whole plants. Fruit from the south side of the south rows, that is, closest to the sprayer, contained slightly more DDT than fruit from the north side of the plants, but the re-

Table 4.—Residues of DDT on plants from each row of a 20-row block sprayed from both sides with air-blast equipment.

Row Position	DDT (p.p.m.)	Row Position	DDT (p.p.m.)
1 (north)	41.34	11	78.57
2	85.66	12	60.92
3	61.62	13	87.34
4	33.81	14	55.19
5	25.70	15	41.26
6	48.10	16	35.23
7	75.91	17	40.80
8	75.42	18	42.81
9	92.71	19	97.44
10	98.24	20 (south)	33.92

Table 5.—Residues (p.p.m.) of DDT on pepper fruit from the north and south side of each row of a 20-row block sprayed from both sides with air-blast equipment at 0 and 7 days after treatment and European corn borer infestation of peppers harvested September 1, 2 and 11.

Row	Fruit from North Side of Plant		Fruit from South Side of Plant		Peppers Infested (%)
	0 Days	7 Days	0 Days	7 Days	
1 (north)	5.8	3.4	3.2	1.4	3.7
2	7.0	5.1	8.4	2.4	4.4
3	4.9	2.3	3.9	1.5	2.1
4	2.7	1.5	5.5	1.2	4.8
5	4.3	1.8	4.5	1.6	4.1
6	4.2	1.8	4.3	1.3	4.1
7	6.1	1.8	3.4	1.6	4.1
8	3.2	2.3	3.7	2.3	1.9
9	5.9	3.0	2.8	2.2	2.3
10	4.3	2.0	3.1	4.2	3.5
11	3.0	2.8	2.1	3.0	1.9
12	6.0	3.4	5.4	2.2	3.5
13	7.8	3.7	7.6	2.8	4.0
14	5.0	1.2	8.5	1.1	2.7
15	4.8	1.9	4.0	1.6	2.5
16	4.9	2.0	4.6	1.9	3.5
17	5.7	2.2	6.5	1.2	6.9
18	4.5	1.6	6.4	2.6	3.3
19	3.8	3.7	9.4	3.2	3.7
20 (south)	4.3	2.3	7.0	3.2	4.6

verse condition was not true on the north side of the field. In general, residues were lower on fruit from air-blast treatments than when the same dosage was applied with the experimental fixed-boom sprayer. The row by row infestation of peppers by corn borer larvae showed little relationship to the intensity of residues except that somewhat better control was obtained with the experimental equipment than with the air-blast sprayer (Burbutis *et al.* 1960).

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Effectiveness of Insecticides Against the European Corn Borer and Green Peach Aphid on Peppers[1]

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Abstract

Four weekly applications of DDT, Sevin® (1-naphthyl methylcarbamate), phosphamidon, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) at 1.0 pound per acre were quite effective in reducing the degree of infestation of second brood European corn borer (*Pyrausta nubilalis* (Hbn.)) larvae to Yolo Wonder peppers. Endrin at 0.5 pound per acre also gave promising results. None of the treatments had any appreciable effect on fruit size or yield. In tests against third generation corn borers, seven applications of a microbial, *Bacillus thuringiensis* Berliner, at 4.0 pounds per acre were ineffective. Dimethoate, phosphamidon, Guthion, endrin, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) held the green peach aphid, *Myzus persicae* (Sulz.), in check whereas a heavy aphid infestation developed in the DDT, Sevin, and Bacillus treatments.

The European corn borer, *Pyrausta nubilalis* (Hbn.), was first found on the Eastern Shore of Virginia in 1931 when four borers were collected as the result of an extensive survey (Jones *et al.* 1939). Since that time this pest has become universally distributed, causing extensive damage annually to field corn, sweet corn, and Irish potato. In addition to these crops the corn borer is constantly expanding its host range and has caused considerable damage to beans, tomatoes, beets, eggplants, and peppers. Peppers in particular have been hard hit and the corn borer has become a limiting factor in the production of this crop on the Eastern Shore. As early as 1949 peppers maturing in July, August, and early September were found to be heavily infested with corn borer. Actually, in one pepper field near Nelsonia, Virginia an infestation of 99.5% was observed in August 1951.

There are three rather well defined generations of the European corn borer on the Eastern Shore of Virginia. Light trap studies have indicated that peak moth emergence of the several generations might be expected to occur as follows: 1st.—May 10; 2nd.—July 1; 3rd.—August 10. Seasonal variation may cause considerable divergence from these dates but in general the peak emergence periods will fall within 7 days of each of the above.

Biological studies have shown that egg-laying activity by the corn borer moths practically ceases after September 7. Consequently, peppers maturing in late September and October are virtually free of borers. Growers have taken advantage of this fact and as a result pepper production on the Eastern Shore of Virginia has been largely limited to fall peppers originating from plants set in late July. This cultural practice has not been entirely satisfactory for three reasons: 1) peppers planted in July have consistently had a poor set of fruit; 2) the production period of a relatively long-lived plant is limited by the late planting; and 3) there has been an increased early

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[2] Entomology Departments, Viriginia Truck Experiment Station, University of Delaware, and University of Maryland, respectively.

Table 1.—Effectiveness of insecticides as foliage sprays against the European corn borer on peppers. Painter, Virginia, 1959.

Treatment and Pounds of Actual Toxicant per Acre[a]	Mature Peppers Infested per 50 Examined[b]		Mature Peppers per 50 Feet of Row[b]	Average Weight per Pepper (Gm.)
	Number	Reduction (%)		
DDT, 1.0	1.3	93.2	91	142
Dibrom, 1.0	15.0	21.1	88	150
Dimethoate, 1.0	4.5	76.3	94	138
Endrin, 0.5	2.3	87.9	85	144
Guthion, 1.0	1.3	93.2	91	147
Kepone, 1.0	6.8	64.2	88	139
Phosphamidon, 1.0	0.3	98.4	97	163
Phosphamidon, 2.0	0.0	100.0	89	154
Sevin, 1.0	0.3	98.4	92	148
Sevin, 2.0	0.5	97.4	97	153
Thiodan, 1.0	5.3	72.1	100	159
Toxaphene, 2.0	9.5	50.0	80	140
Check	19.0	—	89	137
L.S.D. at 5% level	4.2		n.s.	n.s.
at 1% level	5.6			

[a] Sprays applied June 17, 24 and July 1, 8.
[b] Data recorded July 13.

season demand for peppers, both for processing and for fresh market consumption.

The investigations reported in this paper were undertaken as part of an extensive tri-state program to find a practical means of corn borer control in peppers. In addition to the corn borer investigations, observations on the control of the green peach aphid, *Myzus persicae* (Sulz.), were included since a very heavy infestation of this aphid developed in the test area and may have influenced the results to some extent.

MATERIALS AND PROCEDURE.—Two experiments, each consisting of 13 treatments, were conducted at Painter, Accomack County, Virginia in 1959. All insecticides were tested as sprays in the emulsion form except Sevin® (1-naphthyl methylcarbamate) (micro-fine wettable powder), Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one) (wettable powder), and *Bacillus thuringiensis* Berliner (microbial—a Rohm and Hass formulation containing 25×10^9 spores per gram). The test materials were applied in 100 gallons of water per acre at 250 p.s.i. In experiment 1, five Myers Fembro nozzles #6251A (#3 discs) were used per row. One nozzle was placed directly over the center of the row, and 4 nozzles, 2 on each side of the row and spaced 8 inches apart, were directed inward. In experiment 2, because of the increased size of the pepper plants, it was necessary to add a second overhead nozzle in order to insure adequate coverage. The ground speed was 3.2 m.p.h. and 3.5 m.p.h. for the five- and six-nozzle arrangements, respectively. The pepper plots (variety Yolo Wonder) consisted of three rows, each 50 feet long with two untreated rows between each plot. All treatments were replicated four times in randomized blocks and were applied at weekly intervals during critical corn borer moth emergence periods as indicated by light-trap collections.

The relative effectiveness of the different insecticides against the corn borer was evaluated by harvesting all the mature red peppers of marketable size from the middle row of each plot every 7 days. Fifty fruit, selected at random from each plot at each harvest, were checked for larvae. Additional observations were made on the intensity of a green peach aphid infestation which developed in experiment 2. All the aphids were counted in a 2-square-inch area on each of 25 pepper leaves selected at random from each treated plot.

In addition to the screening trials, field observations were made on the general effectiveness of commercial control programs which were adapted as a result of these investigations.

RESULTS AND DISCUSSION.—In experiment 1 (table 1), four weekly applications of insecticides beginning June 17 and extending through July 8 demonstrated that DDT, Sevin, phosphamidon, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate) at 1.0 pound per acre were quite effective in reducing the degree of infestation by second generation corn borer larvae. Endrin at 0.5 lb. per acre also gave promising results. Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Kepone, toxaphene, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*,hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and dimethoate were definitely inferior to the above insecticides, although dimethoate and Thiodan with reductions below the check of 76.3% and 72.1%, respectively, showed promise. None of the treatments had any appreciable effect on the foliage, fruit size or yield. A total of only 0.19 inch of rain fell during the test period.

In experiment 2 (table 2) seven applications of the microbial *Bacillus thuringiensis* Berliner and a superior miscible oil (Sun oil #7) failed to give adequate protection against third generation corn borer larvae. Increasing the rate of dimethoate to 2.0 pounds per acre gave complete corn borer control. The occurrence of smaller fruit in the DDT, Sevin, *Bacillus* and check plots is believed to be associated with a heavy infestation of the green peach aphid which developed in the plots to which these treatments were applied. Significantly larger fruits were harvested from the dimethoate, phosphamidon, Guthion, superior miscible oil, Thiodan, and endrin treatments where apid populations remained at a relatively low

Table 2.—Effectiveness of insecticides as foliage sprays against the European corn borer and green peach aphid on peppers. Painter, Virginia, 1959.

Treatment and Pounds of Actual Toxicant per Acre[a]	Mature Peppers Infested[b]		Average Weight per Pepper (Gm.)	*Myzus persicae*[c]
	Number	Reduction (%)		
Bacillus thuringiensis, 4.0	5.0	46.2	90	3,695
DDT, 1.0	0.5	94.6	105	4,098
DDT, 2.0	0.3	97.8	115	1,928
Dimethoate, 2.0	0.0	100.0	133	10
Endrin, 0.5	0.5	94.6	141	195
Guthion, 1.0	0.5	94.6	154	12
Phosphamidon, 1.0	0.3	97.8	138	27
Phosphamidon, 2.0	0.3	97.8	133	1
Sevin, 1.0	0.0	100.0	90	2,794
Sevin, 2.0	0.5	94.6	107	3,250
Superior oil, 1 gal.	7.0	24.7	136	225
Thiodan, 1.0	2.5	73.1	143	21
Check	9.3	—	84	3,000
L.S.D. at 5% level	1.8		20.9	439.1
at 1% level	2.4		28.0	588.3

[a] Sprays applied July 23, 30 and August 3, 10, 17, 24, 31.
[b] Per 50 examined September 4.
[c] Per 25 leaf samples (each 2 sq. in.) examined August 31.

level. None of the insecticides caused any noticeable phytotoxicity to the fruit or foliage.

It will be noted that a total of four applications was made against second generation corn borer larvae (table 1) as compared with seven for third generation larvae (table 2). These additional applications were necessary because of the extended emergence period of third generation corn borer moths. Instead of reaching a definite peak and then declining rather rapidly in an overall period of about 3 weeks, third generation moth emergence started July 26 and continued at a rather uniform and low rate through September 7. The reason for such a spread is not known but it does point out the value of light traps or other life history studies in keeping a close check on seasonal insect activity and in detecting unusual variations. These variations can and do result in economic loss to those following a predetermined control schedule based on a "normal" year.

The degree of reduction in borer damage through weekly applications of DDT (tables 1 and 2) was considerably higher than the 62% reduction obtained by MacCreary & Connell (1958) in Delaware with five applications spaced at approximately 2-week intervals.

Based on the results obtained in experiment 1, DDT was recommended for commercial use for corn borer control on late peppers in Virginia. Observations on commercial control were entirely satisfactory with both airblast and high-volume-boom sprayers although it was slightly better with the latter. DDT dusts were definitely not as effective as the sprays and are not recommended. In one field where both dusts and sprays were used, approximately 25% of the peppers were infested in the dusted area as compared with only 4% in the sprayed section.

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Selection Response and Genetics of Parathion Resistance in the Pacific Spider Mite, *Tetranychus pacificus*[1]

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ABSTRACT

Parathion resistant and parathion susceptible strains of the Pacific spider mite, *Tetranychus pacificus* McG., were derived from certain field populations and established as laboratory colonies.

The two colonies were characterized by dosage-mortality curves for the effect of parathion and also Aramite® (2-(*p-tert*-butylphenoxyl)-1-methylethyl 2-chloroethyl sulfite).

One generation selection experiments for parathion resistance and for Aramite resistance were performed on the susceptible colony. The colony responded to both acaricides. The colony responded to selection for parathion resistance by not only an increase in LC-50 but also by producing a population partitioned into susceptible and resistance classes, thus providing evidence for the existence of a major gene for resistance.

The latter genetic hypothesis was then tested by performing conventional crosses between the original susceptible and resistant colonies. The results of these crosses produced further strong evidence for a major mendelian dominant with resistance to parathion dominant to susceptibility.

The development and occurrence of resistance to various acaricides in several members of the mite family Tetranychidae is continuing to prove itself a problem not only to the grower and pest control operator but also to the research worker. It has re-emphasized the complexity of the organism with which he is dealing and the difficulties that are inherent in regulating their numbers. The solution of this problem will require not only the development of basic information regarding resistance but also basic biological and ecological studies of the organisms involved. Herein are presented the results of several tests designed to increase the understanding and delimitation of the mite resistance problem. The work consisted of a brief selection program to develop a resistant mite strain from a field-collected susceptible strain and the mating of resistant and nonresistant mites to study the mechanism by which resistance is inherited.

MATERIALS AND METHODS.—In the summer of 1956 several strains of the Pacific spider mite, *Tetranychus pacificus* McG., one from cotton in the San Joaquin Valley suspected of resistance to organophosphorous compounds and several from alfalfa fields grown for seed in which no control difficulties had been encountered, were brought into the laboratory and maintained separately on Henderson dwarf lima bean plants. On the basis of preliminary tests the mites from the several fields of alfalfa were found to have a similar LC-50 response to parathion and thus were consolidated into one colony. The dosage mortality curves of this colony and the one suspected of resistance collected from cotton were determined in the following way. Infested leaves were selected from the colonies and pinned onto the primary leaves of young bean plants with roots placed in bottles of water. After the mites had transferred to the new plants (14 to 16 hours) these leaves were removed and the petioles of the newly infested leaves banded with lanolin. These infested leaves along with uninfested plants bottled in a similar manner were then carefully dipped in the test solution

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[2] The authors wish to acknowledge the help of Mr. Roy L. Hale during the laboratory phase of the work.

[3] Alkylated aryl poly-ether, manufactured by Rohm and Haas Co., Philadelphia, Pennsylvania.

Table 1.—Parathion dosage-mortality response of original field-collected susceptible (S) and resistant (R) colonies of *Tetranychus pacificus*.

Mortality in Check (%)		Parathion Dose (Per Cent Solution)											
		0.0001	0.0003	0.0005	0.00075	0.001	0.0015	0.0025	0.0035	0.03	0.05	.1	.3
		Susceptible—S											
3.4	Mortality (%)[a]	2.7	43.7	71.3	87.2	93.2	96.1	98.9	95.5				
	No. tested	250	250	550	300	850	600	300	300				
		Resistant—R											
3.1	Mortality (%)									3.3	20.9	61.5	98.9
	No. tested									175	175	225	210

[a] Corrected for natural mortality.

and allowed to dry. Immediately upon drying, healthy adult female mites from the infested leaves were transferred to the also treated uninfested leaves. The number of females transferred were recorded. These plants were held 24 hours at 80° to 85° F. after which the number of live mites remaining were counted. Each dosage was replicated on two to four leaves and was repeated on several different days. The results of these tests appear in table 1, represented by the average mortality over all leaves and days, corrected for natural mortality. This information is also graphically presented in figure 1. Hereafter the susceptible and resistant strains will be referred to as S and R, respectively.

The solutions used in these tests were prepared by mixing an amount of technical parathion, 99.9%, or Aramite®, 90.5% (2-(*p-tert*-butylphenoxyl)-1-methylethyl 2-chloroethyl sulfite) with a given amount of water plus 2 drops of Triton X-100[3] in a Waring Blendor for several minutes. This solution was then aliquoted into a series of beakers containing an amount of water to produce the appropriate dilutions. Two drops of Triton X-100 were added to each beaker per 100 cc. of dilutions. The check solution contained only Triton X-100 and water. All solutions were prepared just prior to testing.

The selection experiments were carried out as follows: Several thousand female mites from the S colony were transferred to bottled plants and treated in the previously described manner. Several hundred of these mites were transferred to uninfested leaves immediately following treatment to estimate the per cent mortality while the remainder were left on the treated plants. After 24 hours, the adult female survivors were selected and placed in a new colony. The progeny of these surviving females was later tested for their dosage-mortality response to the selecting chemical. The dosages used for selection were based on the original dosage mortality curve of the S colony.

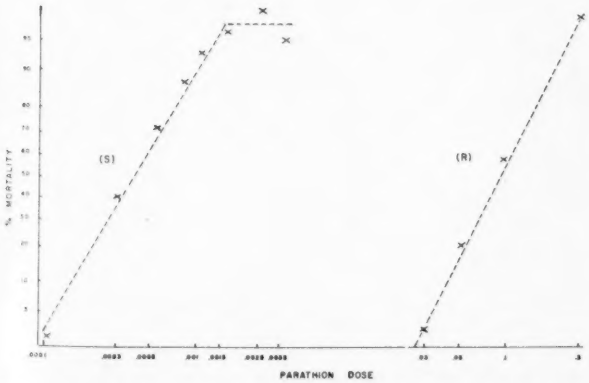

FIG. 1.—Dosage-mortality curves of field-collected resistant (R) and susceptible (S) strains of *Tetranychus pacificus* in response to parathion.

The crossing experiments were conducted as follows: Reciprocal crosses between S and R colonies were made by placing together a single female deutonymph with two males of the opposite strain on a portion of excised bean leaf maintained on wet cotton. After the females had emerged and mated each mite pair was transferred to the leaflet of a bottled bean plant isolated with a band of lanolin around the petiole. After 3 to 5 days of egg laying the females were removed to new leaflets. The parent females lived about 8 to 12 days and produced approximately 30 to 40 female young. The F_1 progeny were allowed to develop on the leaflets until sufficient females had matured to permit testing. The female progeny from each parent cross were then divided into three or four groups, each group being placed on one of two primary leaflets of a bottled bean plant. These mites were permitted to feed 14 to 16 hours after which both the infested and uninfested leaflets in each pair were treated in the previously described manner. Upon drying, the surviving adults were transferred from the infested to the uninfested leaflet in each pair and held 24 hours. The number of live females was then counted. It was observed that adult females 2 to 3 days old were optimum for testing. Backcrosses were made between F_1 females placed with males from the S colony. Again the progeny of each female was maintained separately and handled as above. The dosage mortality curves of the F_1 and backcross female progeny were then determined. A conventional F_2 progeny is difficult to obtain since this is an arrhenotokous organism.

RESULTS AND DISCUSSION.—*Statistics.*—The dosage-mortality information of the original S and R populations (table 1; fig. 1) was examined statistically by calculating a number of heterogeneity chi squares to determine the reliability of the data produced by the above techniques. Leaf to leaf replicates for a given day and dose were found to be homogeneous while a small amount of heterogeneity was detected operating between days for a given

Table 2.—Parathion dosage-mortality response of various populations of *Tetranychus pacificus* resulting from selection and crossing experiments.

Colony	Mortality in Check (%)		Parathion Dose (Per Cent Solution)											
			0.0001	0.0003	0.0005	0.001	0.003	0.005	0.01	0.03	0.05	0.1	0.2	0.3
Selected for resistance. Survivors of one treatment, 99% mortality dose.	12.4	Mortality (%)[a]	0	14.9	41.8	60.8	64.0	66.9	49.4	44.5	52.6	71.8	96.1	98.6
		No. tested	100	200	300	300	200	100	300	675	200	575	500	375
Selected for resistance. Survivors of one treatment, 90% mortality dose.	10.8	Mortality (%)		3.8	19.3	61.3	72.3	77.6	58.5	68.2	81.5	91.7		100
		No. tested		200	200	200	300	100	300	275	200	175		100
F_1 ♀ progeny from R ♀ ×S ♂	4.4	Mortality (%)							15.7		47.7	92.3		
		No. tested							72		74	68		
F_1 ♀ progeny from S ♀ ×R ♂	4.9	Mortality (%)							17.7		19.9	92.6		
		No. tested							46		42	43		
Backcross of F_1 ♀ from R ♀ ×S ♂ parents with S ♂	8.9	Mortality (%)	7.2	30.9		49.3			46.5	46.3	62.2	92.6		
		No. tested	71	70		46			39	45	32	30		
Backcross of F_1 ♀ from S ♀ ×R ♂ parents with S ♂	7.0	Mortality (%)	0	46.9		53.5			58.5	58.1	77.7			
		No. tested	32	81		74			57	41	29			

[a] Corrected for natural mortality.

dose. The data were then combined and fitted to the logistic function, and good fits were obtained. The S population (excluding the two highest doses) yielded a chi square testing goodness of fit equal to 2.14 which having 4 degrees of freedom corresponds to a probability of approximately 0.74. The same chi square for the R population equaled 2.81 which with 2 degrees of freedom corresponds to a probability of approximately 0.25. Thus, considerable reliability can be placed on a curve, the points of which were determined on the same day. On the other hand, because of the day to day heterogeneity, small discrepancies between experiments done at different times should be viewed with caution.

Selection Experiments.—Two single selections of resistant individuals from the S colony were made with parathion, one at the 90% and the other at the 99% mortality level. The progeny from these survivors were maintained in separate colonies and their dosage-mortality response determined (table 2; fig. 2).

Both intensities of selection produced populations clearly composed of two discrete classes: individuals as susceptible as those of the S colony and individuals apparently as resistant as those of the R colony.

Estimating from the level of the plateaus, selection at the 90% mortality level produced a population with approximately 34% resistant individuals while the more intense selection (99% mortality) produced a population with approximately 46% resistant individuals.

The abrupt appearance of these two classes suggests that the selection process has either brought into high frequency a major gene for resistance which was already present or possibly created a high probability of occurrence of a simple gene combination capable of conferring high resistance. The former possibility is made more likely by the fact that the original S colony showed signs of possessing some rare individuals of unusually high resistance (figure 1, see responses to doses 0.0025 and 0.0035).

The results of a single selection for Aramite resistance are shown in figure 3, and table 3. In this case rather than a partitioning of the population into distinct classes there appeared to be a general increase in resistance accompanied by a possible increase in individual variability. The fact that there was a response to selection indicates that Aramite resistance has a genetic basis but the data give little reason to believe that individual resistance is not continuously distributed and under polygenic control.

Crossing Experiments.—As already indicated, families from each mating pair were tested separately. However, because of the small number of individuals per family it was found that little information would be lost by combining the data. This procedure was justified when for most doses and most crosses no interfamily heterogeneity was found.

F_1 females from both reciprocal crosses showed LC-50's in the general region of the parent R population. This would suggest the near complete dominance of resistance over susceptibility.

Backcrossing the F_1 females with susceptible males (table 2; fig. 4) gave a clear partition of the backcross progeny into resistant and susceptible classes. Consider-

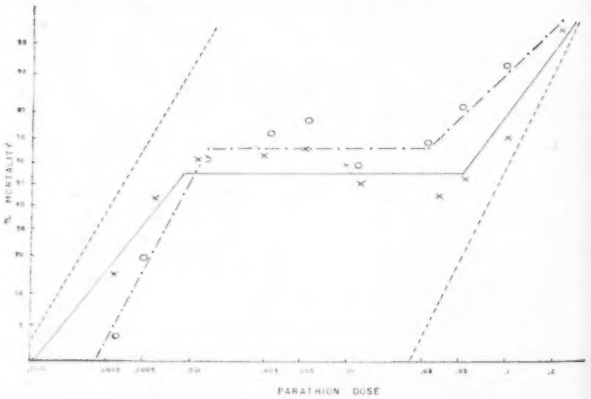

FIG. 2.—Dosage mortality curves of progeny derived from mites of the S colony which had survived with parathion at the 90% mortality level (o and -·-·-·-) and 99% mortality level (x and ———). Dashed lines - - - - represent original R and S strains.

Table 3.—Aramite dosage-mortality response of the original parathion-resistant colony and of an Aramite resistance selected colony of *Tetranychus pacificus*.

Mortality in Check (%)		Aramite Dose (Per Cent Solution)										
		0.00075	0.001	0.0015	0.0025	0.0035	0.005	0.0075	0.01	0.025	0.035	0.05
		Susceptible—S										
9.8	Mortality (%)[a]	0	2.8	6.9	58.2	91.9	93.3	99.9				
	No. tested	200	300	200	600	300	400	400				
		Selected for resistance survivors of one treatment 99.5% mortality dose										
13.9	Mortality (%)		1.3	11.7	47.3		77.0	73.9	84.3	96.5	97.1	100
	No. tested		100	100	500		600	600	400	600	200	100

[a] Corrected for natural mortality.

ing the values from the plateaued part of the curves, one backcross gave 46.8% individuals in the susceptible class and the other backcross gave 56.2% individuals in the susceptible class. Neither of these values differs significantly from 50%. These two facts, the partitioning of the backcross progeny into two classes and the incidence of the two classes approximating 50%, provide strong evidence that resistance is under the control of a major mendelian dominant.

The rather pronounced (significant) non-linear arrangement of the F_1 points suggests that in addition to this dominant factor, there may be some sort of modifying factor or factors involved. This disturbance could easily be explained by the segregation of modifiers in the S population which only act in the presence of the major gene for resistance. For example, figure 5 shows one of many model systems of modifier-major gene interaction which would yield a set of theoretical curves giving good fits to all the empirical curves of the crossing experiments. Elucidation of such systems would require further experimentation such as the establishment and genetic investigation of a series of inbred lines derived from the S population. The question of modifiers, however, should not obscure the fact that there still remains good evidence for a single major gene for resistance.

In some previous studies of the genetics of resistance it has been the practice to choose a single dose, which would give a high mortality in the susceptible stock and a low mortality in the resistant stock, and then apply this single dose to F_2 and backcross progeny. Taylor & Smith (1956), using this technique with *Tetranychus telarius* (L.) obtained mendelian ratios from the crossing of resistant and susceptible mites, similar to those reported here. It should be emphasized, however, that the production of mendelian ratios by a single dose is necessary but by no means sufficient evidence for a major gene. The dose used could well be truncating a continuous distribution of resistance at levels numerically equivalent to mendelian ratios.

Therefore, it is also necessary to demonstrate that the

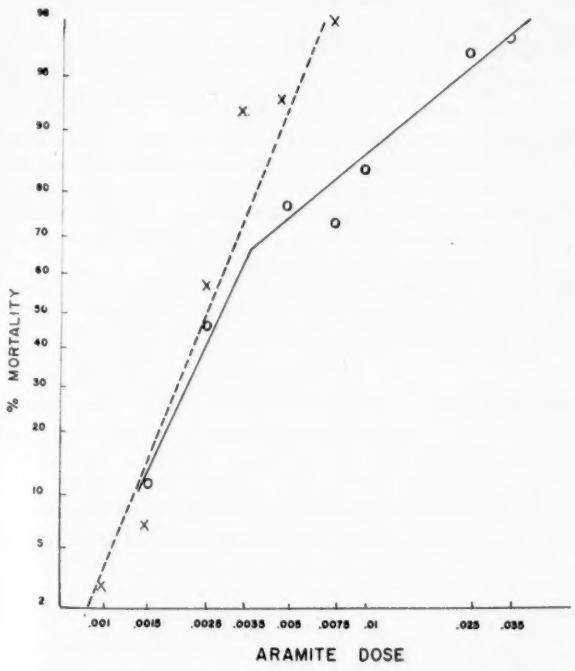

FIG. 3.—Dosage-mortality curves of susceptible (S) mites and the progeny of survivors of a dose of Aramite equivalent to the 99.5% mortality level. x and – – – – represent (S) strain, and o and ——— represent selected strain.

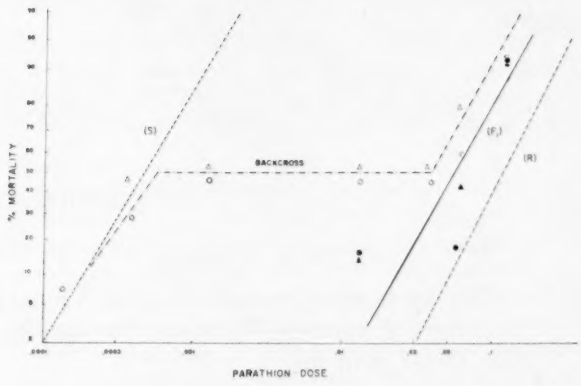

FIG. 4.—Dosage-mortality curves of progeny from following crosses.

F_1, R♀×S♂ ▲ ———
F_1, S♀×R♂ ●
Backcross, F_1 (R♀×S♂)×S♂ ○ – · – · –
Backcross, F_1 (S♀×R♂)×S♂ △
P_1's, R♀ and S♀ – – – –

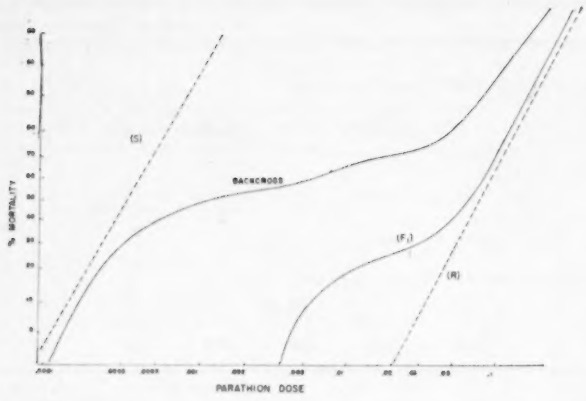

FIG 5.—Theoretical curves of P_1, F_1 and backcross results expected assuming a major gene for resistance (R, r) and a hypostatic modifier (M, m) which acts such that R-, mm has full resistance R-, M- has a lower level of resistance and any other genotype is susceptible. Further assumptions necessary for fitting to data were: Resistant strain homozygous RR, mm and susceptible strain homozygous rr but segregating for M, m with frequency of M equal to 0.20.

segregating progeny (backcross or F_2 where possible) is partitioned into discrete resistance classes and for this purpose at least four appropriately chosen doses (rather than one) are required for establishing the existence of a plateau in the dosage-mortality curve. The demonstration of both discrete classes and the occurrence of these classes in mendelian proportions as has been done in this case may then be taken as good evidence for simple mendelism. However, such information is still not sufficient, for as Crow (1957) has indicated, a polygenic system underlying a phenotypic threshold could "mimic" simple mendelism.

Thus, it appears that the determination of the exact mechanism of inheritance in the material reported here as well as in the work of some others will require still further experimentation involving not only studying inbred lines as already suggested but also possibly a system of backcrossing as suggested by Crow (1957, p. 234).

Finally it might be mentioned that the above results considered in connection with others may constitute a case contrary to the current view that different populations of the same species or of different species will respond with different genetic mechanisms when subjected to selection for the same phenotype (King 1955). In the work reported here on *T. pacificus*, a single mendelian dominant for resistance has apparently arisen twice: once "naturally" in parathion-treated cotton fields and once by artificial selection in the laboratory. In addition the work of Taylor & Smith (1956) indicates parathion resistance in a different species, *T. telarius*, may also be under the control of a simple mendelian dominant and the inference that this is the same gene is given some support by the fact that a comparison of LC-50 values indicates the same magnitude of resistance in the two species. Also the work on another species, *T. atlanticus* McG., by Andres & Reynolds (1958) indicates the same magnitude of resistance. In this latter case, however, no genetic evidence is available.

Similar findings have been obtained by Kikawa (1958) in his studies of *Drosophila* where parathion resistance is apparently under the control of the same simple mendelian factor in strains of such diverse origin as Japan and Sweden.

The above genetic evidence indicates that future biochemical investigations of the mechanism of parathion resistance might possibly reveal a wide ranging system of homologous genes such as that being found for insect eye pigment synthesis (Ward & Hammen 1957).

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Aerial Forest Spraying Against Spruce Budworm—A Problem of Mutual Interest in Canada and the United States[1]

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Abstract

By far the largest amount of aerial forest spraying in both Canada and the United States has been against the spruce budworm (*Choristoneura fumiferana* (Clem.)). Although initial development of the method was based largely on cooperative trials, subsequent full-scale operations in the two countries have been carried out largely on opposite sides of the continent: in the Atlantic Region of New Brunswick and Quebec in Canada and in Pacific Northwest and Rocky Mountain Regions of the U.S.A. The paper reviews recent Canadian and United States operations from the point of view of differences in host forests and outbreak behavior, spraying policy, techniques and effectiveness. Particular reference is made to the problem of damage to Atlantic Salmon populations in New Brunswick and to current sets of the feasibility of reducing DDT concentration to minimize adverse effects while maintaining acceptable pest population reduction.

Recent statistics from the Division of Forest Pest Control of the United States Forest Service list 11 forest insect pests combatted by the aerial application of DDT since this insecticide became available in commercial quantities some 15 years ago (Benedict 1959). This is exclusive of about 10 million acres of spraying for control of the gypsy moth (*Porthetria dispar* (L.)) carried on during the period in various parts of New England, New York, New Jersey, Pennsylvania, and Michigan. Operations against other forest insects to 1958 total over 10½ million acres mostly at an emitted dose of 1 pound DDT per acre.

In Canada the method has been used against four forest insects (Webb 1959). Three of these, the black-headed budworm (*Acleris variana* (Fern.)), the hemlock looper (*Lambdina fiscellaria* (Guen.)), and the spruce budworm (*Choristoneura fumiferana* (Clem.)) occur on both lists but it has been the last-mentioned of these species that has been responsible for by far the most extensive operations on both sides of the border. Of the total against all forest insects in the United States, 9.7 million acres, or 92% have been treated against this widely distributed and excessively destructive native pest. Coincidentally, approximately the same amount of DDT has been used to date against this insect in Canada, between 9 and 10 million pounds. This represents an even larger proportion of the total for all insects than in the United States, almost 99%. In Canada, however, almost all of this has been applied at an average emitted dose of ½ pound DDT in ½ U. S. gallon of oil-solvent per acre on a gross operational acreage of almost 18 million acres.

Cooperative Early Development.—It will be obvious from the foregoing statistics that the development of the aerial application method against forest insects on this continent has so far been chiefly influenced by the requirements of this particular pest problem. Because of a common and urgent interest in the problem much of the early developmental work relating to dosage and methods of application was undertaken cooperatively by United States and Canadian investigators. In 1945, representatives of the U. S. Bureau of Entomology and Plant Quarantine and the U. S. Fish and Wildlife Service took an active part with Canadians in the consultative and observational stages of the first full-scale spraying operations against this insect, carried out by the Ontario Department of Lands and Forests in the Lake Nipigon area. In the same year, joint tests relating chiefly to dosage and timing were carried out by the Bureau of Entomology and Plant Quarantine personnel in collaboration with Canadian Provincial and Federal investigators in Algonquin Park, Ontario, and Kabonga Provincial Park in Quebec. Joint field tests were again carried out in 1951 in the Bagotville area of Quebec, in this case involving collaboration between the United States Bureau, the Quebec Bureau of Entomology and the Maine Forest Service. In retrospect it is clear that these joint investigations played a major role in establishing the 1 pound DDT in 1 gallon of formulation per acre as a more or less standard empirical dose for forest spraying, and for determining the optimum stage in the life cycle at which to apply the insecticide. Further tests of these aspects of the problem as they applied to conditions in the Pacific Northwest were begun by United States workers in Oregon in 1948 and work relating especially to problems of dispersal apparatus was carried on for several years at the Agricultural Research Center, Beltsville, Md. On the Canadian side investigations were continued in 1951 and 1952 in the Kenora area of Ontario by the Division of Forest Biology of the Federal Department of Agriculture in collaboration with the Defense Research Board. Among the more important results, these intensive studies demonstrated the screening effect of foliage on a drifting spray cloud, the closer dependence of mortality on droplet density than on spray volume or droplet size and something more of the relation between spray effectiveness and the stage of larval development at which the insecticide is applied.

Recent United States-Canadian Operations.—*Differences in Host Forests and Outbreak Dynamics.*—Although sharing a more or less common experimental beginning, subsequent full-scale use of the aerial method in the two countries has been characterized by important differences in policy, technique, and results. The chief reason for this is that United States and Canadian operations have been carried out almost entirely on opposite sides of the continent in distinctly different forest types

[1] Contribution to a symposium on "Insect Control Problems of Mutual Interest to Canada and the United States" at Joint Annual Meeting, Entomological Society of America and Entomological Society of Canada, Detroit, Michigan, No. 30–Dec. 3, 1959.

Contribution No. 616 Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada. Accepted for publication February 8, 1960.

and conditions. United States operations since 1949 have been carried out chiefly in Douglas fir—true fir—Engelmann spruce forests of the Pacific Northwest and Northern Rocky Mountain Regions. (Smaller operations have been carried out as far south as New Mexico and in the Northeast in northern Maine). Except for the pioneer operations in Ontario in 1945–46, Canadian budworm spraying has been carried out in spruce-balsam forests of northern New Brunswick and Lower St. Lawrence-Gaspé areas of Quebec.

No exact comparative study has yet been made of the outbreak dynamics of eastern and western forms of this insect but it is virtually certain that important differences do occur and that these are reflected in some degree in the difficulty of dealing with the problem in the respective areas and in the effectiveness of the spray operations. Obviously of prime importance are the differences in host forests. Although occupying very large areas *in toto*, susceptible western forest types, particularly in mountainous areas, do not compare in uniformity or continuity with the spruce-fir type of Eastern Canada. Of the two principle hosts, Douglas fir in the West appears to be better able to withstand prolonged severe attack than balsam fir in the East, and may compare more closely in this respect with the other important eastern host, white spruce. In combination these factors help to explain the more limited extent of western outbreaks compared with those in the East, and the lack of an equivalent history of periodic widespread forest damage.

Differences in Spraying Policy.—As might be expected, differences in the basic problem have been largely responsible for the development of certain differences in the two countries in spraying policy and technique. Policy has differed chiefly with respect to the relative emphasis placed on tree protection and outbreak suppression. In the United States, spraying has generally been recommended relatively early in the outbreak with the dual objectives of forestalling tree mortality where this might occur within 1 or 2 years, and of minimizing the threat of reinvasion by spraying adjacent buffer zones of lighter attack. Under special circumstances in the Pacific Northwest, light infestations have been treated to give early protection to especially high-value timber stands or to prevent the weakening of trees in danger of bark-beetle attack (Whiteside 1958). In Canada, a more conservative policy has been followed of spraying only in areas of high hazard where a further year's attack would seriously threaten the life of trees. To some extent this policy has been dictated by operational and economic limitations. In 1957, for example, operations of 6.5 million acres, requiring by far the largest aerial fleet and facilities ever assembled for the purpose, covered less than half of the total area of moderate to severe current attack in the region that year. Apart from this practical aspect, however, the policy has been considered the safest to adopt under Eastern Canadian conditions to give the best assurance of avoiding unnecessary spraying and to minimize adverse effects. A limitation of this policy, however, is that it calls for a high degree of accuracy in measuring hazard and keen judgment in assessing the ability of trees to withstand attack. It also involves a high risk of failure in the event of inadequate treatment.

Differences in Spraying Techniques.—The most important aspects in which United States and Canadian techniques have differed in recent years is in respect to dosage and timing of applications. United States operations, aiming chiefly at maximum population reduction, have continued to use the conventional 1 pound per acre dosage applied relatively late in the feeding period to obtain maximum kill of larvae. This approach was also used in the first year of operations in New Brunswick. Since 1953, however, timing has been altered to give increased emphasis to the preservation of the current foliage crop by commencing spraying somewhat earlier in the season. Although sometimes involving a sacrifice in percentage of kill, this approach has the advantage in large operations of extending the time available for spraying. Since 1953, dosage on Canadian operations has been halved to give an average emitted dose of $\frac{1}{2}$ pound DDT in $\frac{1}{2}$ U. S. gallon of oil solvent per acre. Adopted principally for reasons of economy and efficiency, to allow the treatment of approximately twice the area with the same aircraft fleet and facilities, this dosage, carefully applied, has been found to give satisfactory results in terms of tree protection and has had the added advantage of being less damaging to other forms of life.

EFFECTIVENESS OF UNITED STATES-CANADIAN OPERATIONS.—Reports on results of the major operations, although indicating generally satisfactory results in both countries also reflect the differences in basic outbreak dynamics between East and West. Oregon-Washington operations, for example, covering nearly 5 million acres up to 1959 have resulted in "remarkable recovery" of defoliated trees and have restricted timber losses to less than 10,000 acres (Whiteside 1958). The policy of spraying to eliminate infestation as well as to prevent damage appears to have been justified from the fact that less than 2% of the areas have required respraying because of reinfestation (Furniss 1959). Successful achievement of objectives has also been claimed for the New Brunswick-Quebec operations. Except in some relatively small areas where spraying was ineffective owing to late timing or poor application, severe mortality of both balsam fir and spruce was forestalled through the course of a full-scale outbreak in largely mature forests totalling over 9 million acres. In contrast to the western experience, however, reinfestation proved a very difficult problem. This was the combined result of rapid resurgence of residual budworm population left after spraying reinforced by recurring large-scale moth invasions from surrounding unsprayed areas in the two Provinces and northern Maine. Because of this, in the 7 years' operations until the outbreak declined from natural causes over half of all areas were sprayed more than once and small parts of the original outbreak epicenter in northern New Brunswick were resprayed three and four times.

EFFECTS ON NATURAL CONTROL FACTORS.—There is yet no evidence from biological studies of these large operations of serious damage to the natural controls of the pest. The outbreak in northern New Brunswick, which has been studied intensively in relation to this aspect of the problem, appears to have lasted approximately the same length of time as the previous one in the region despite extensive spraying and respraying and the

preservation of a highly-susceptible forest condition.[2] Studies of effects on parasites in both the East and the West have thus far failed to show that any species has been eliminated. On the other hand, at least one important parasite species has shown consistently increased percentage effectiveness following spraying when compared with unsprayed populations (Macdonald 1959).

No evidence has yet been found in Canadian operations of increased incidence of other pests from the use of DDT. An exception to this in the United States occurs in the case of Montana operations where the use of DDT against spruce budworm in 1956 and 1957 is believed to have encouraged an outbreak of the spruce spider mite (*Oligonychus ununguis* (Jac.)) on Douglas fir (Johnson 1958).

ADVERSE EFFECTS ON FISH.—A problem of particularly urgent current concern in Canada is that of adverse effects against fish. Conclusions from earlier experimental work on inland game species indicating that a properly-applied dosage of 1 pound DDT per acre would be unlikely to cause serious lasting damage have proved to be over-optimistic for Atlantic Salmon affected by spruce budworm spraying in New Brunswick and Quebec, and for Coho and other species of Pacific salmon affected by a black-headed budworm operation on Vancouver Island in 1957. Fisheries investigations in affected streams in both areas have revealed drastic losses of the youngest age classes of these species, even at the reduced dosage of ½ pound DDT per acre used in the East. It has been predicted from the New Brunswick studies that this damage will be reflected in serious reductions in the number of adults returning from the sea over the next few years (Kerswill 1958). Severe, and to some extent lasting damage has also been shown to occur to aquatic insects forming the bulk of available fish-food. This problem has been aggravated by the persistence of reinfestation and the necessity of treating most of the New Brunswick salmon watersheds on two or more occasions.

A direct result of mounting concern over the salmon problem in Canada has been the formation of a Federal interdepartmental committee representing agencies concerned with forest insect investigations and forest, wildlife, and fisheries resources (Prebble 1960). The function of the committee is to review proposals for forest spraying in Canada, particularly those in which conflicts of interest arise, and to assess the need for research into particular aspects of the problem. As a first result, collaborative tests of alternative insecticides and formulations that might be less damaging to salmon were undertaken in 1958 and 1959 by the Division of Forest Biology and Fisheries Research Board with assistance from Forest Protection Ltd. representing New Brunswick and the pulp and paper industry of the Province. Results to date have been reviewed by Fettes (1960). Of other insecticides known to be less toxic to aquatic life, none has yet been found that can match the effectiveness of DDT at light dosages against forest defoliators. However, considerable promise for minimizing adverse effects has been obtained from tests of reduced concentrations of DDT. These suggest that a concentration one-half that of the dosage hitherto used in New Brunswick and Quebec (½ pound DDT in ½ U. S. gallon formulation per acre) with adequate droplet coverage may be equally effective against the spruce budworm while being less damaging to aquatic life. It is anticipated that the half-strength dosage (¼ pound DDT in ½ U. S. gallon formulation per acre) will be tested at full operational scale against a resurgent outbreak in central New Brunswick in 1960.

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[2] Since this paper was accepted for publication, the following information has been obtained: Although patches of vigorous attack remain in more-recently infested areas of central New Brunswick, the outbreak is currently at or near endemic levels in northern parts of the Province.

Relation of DDT-Irritation Threshold to Knockdown of Three Species of Anopheline Mosquitoes[1]

Otto Hecht, Ofelia Mancera, and Alfredo Barrera[2]

Abstract

The threshold of irritation was determined for bloodfed and unfed females of *Anopheles albimanus* Wiedemann, *A. aztecus* Hoffmann, and *A. quadrimaculatus* Say taken from laboratory colonies. By means of a special device each mosquito which flew off from the DDT-treated surface was trapped in a container, where it remained in almost continuous contact with the insecticide until knockdown occurred. When the exposure times which provoked a response to irritation (thresholds) were compared with the exposure times resulting in knockdown, no correlation was found between the records for the same specimen. But summarizing all the observations (made 5 times with 20 mosquitoes of each species, with bloodfed or unfed females) the collective results indicate that the most irritable species, *A. albimanus*, is also the most susceptible one to the knockdown effect.

The comparison of the thresholds of irritation between unfed and bloodfed females showed that nourishment influenced the various species to a different degree. Among *A. aztecus* almost no decrease of irritability was caused by the replete stomach, whereas bloodfed *A. albimanus*, as well as bloodfed *A. quadrimaculatus*, were approximately one-half as irritable as unfed females.

A detailed knowledge of the irritation of mosquitoes at contact with DDT-covered surfaces is valuable for the understanding of the deterrent action of the insecticide.

In an earlier paper (Barrera *et al.* 1959) we referred to experiments concerning the irritability of *Anopheles albimanus* Wiedemann, *A. aztecus* Hoffmann, and *A. quadrimaculatus* Say, unfed as well as bloodfed females. When we introduced 20 specimens into a transparent plastic cylinder the ends of which were covered with DDT-impregnated paper, the irritated mosquitoes flew from one end to the other, almost never settling on the smooth plastic material; the flights were counted over a long period and their numbers taken into account as the measure of irritability. The mean minimum irritative dose DMI (=*dosis pro medio minima irritativa*) was calculated for each group and thus the differences in irritability between the species and differing physiologic conditions were ascertained.

The object of the present study was to determine the threshold of irritation for the three species, bloodfed and unfed, and furthermore to define for each lot of mosquitoes the time of exposure to the insecticide necessary to produce knockdown as an approximate measure of their susceptibility to the toxic (lethal) effect of the insecticide, in order to compare the irritability of each species and the physiologic condition with its susceptibility.

Technique.—The device (fig. 1) used for this purpose was referred to by Hecht *et al.* (1958). It consists of a truncated pyramid (a) and of small box-like receptacles (e) which can be attached to the open top side (c) of the horizontally situated pyramid.

The four walls of the pyramid are made of transparent plastic material, the four edges being 12 cm. long. Its square base (11 cm.×11 cm.) was made of cardboard (b) lined with paper impregnated with a 4% DDT solution in Risella oil, corresponding to 1.428 gm. of DDT/$m.^2$, or in the controls with Risella oil paper free of insecticide.[3] The open top side (4 cm.×4 cm.) of the pyramid (c) is provided with a double wall cardboard frame (d), which serves as a guide for plastic slide (h).

The long walls (8 cm.×4 cm.) of the box-like receptacles (e) are made of transparent plastic, their square ends (4 cm.×4 cm.) are fitted similarly with double wall cardboard frames (f, g) which serve as guides for plastic slides (i, j) lined with DDT-impregnated paper, or, for use in the controls, with Risella oil paper free of insecticide.

One of these boxes is attached to the open top side of the horizontally placed pyramid, their cardboard frames held together by bulldog clips (k, k[1]). Removing simultaneously the slide at one end (i) and that of the pyramid (h), connection with the pyramid was established. The other end of the box remains closed by a bobbinet (l) attached to a window in the cardboard frame, even if the corresponding slide (j) is removed.

Method of Observation and Evaluation.—We used, as in the earlier study, 2-day-old females taken from laboratory colonies, separately unfed ones and others that had fed on a rabbit during the early hours of the preceding night. At the beginning of a test 20 mosquitoes were introduced into the pyramid through a hole in the slide (h) on the top side. The mosquitoes settle and rest on the paper at the base (b) of the pyramid. One of the rectangular plastic containers (e) was attached to the small end of the pyramid and the slides (h, i) between the pyramid and the container were pulled away. As soon as a mosquito had passed into the container, the slides were lowered, the container with its single mosquito was removed and substituted by an empty one. We registered the time each one of the 20 mosquitoes needed to leave the DDT-surface and to enter the reception box; in this manner we determined the time each specimen took to produce an irritation response.

The mosquitoes singly trapped in the box-like containers avoided settling on the smooth plastic surfaces and rested on the DDT-paper of the lowered slides (i, j), therefore they remained in almost continuous contact with the insecticide until they were knocked down. By registering the time of knockdown for each of the 20 speci-

[1] Presented in part at the Joint Annual Meeting of the Entomological Societies of America, Canada, and Ontario held at Detroit, Michigan, November 30 to December 3, 1959. Accepted for publication February 12, 1960.

This study was carried out as part of the research program of the National (Mexican) Commission for the Eradication of Malaria. The opinions of the authors expressed in this paper are not necessarily those of the Commission.

[2] Sección de Entomologia, Dirección de Investigación y Fomento. Comisión Nacional para la Erradicación del Paludismo, S.S.A., México, D. F.

[3] The way to prepare these sheets of insecticide papers, which are provided by the Division of Environmental Sanitation of the World Health Organization, apparently obtains a very uniform distribution of the particles of DDT, their firm adherence to the substrate, and consequently the possibility of using them many times over a rather long period.

Table 1.—Exposure time in minutes needed to provoke a response to irritation.

Species of Anopheles	Unfed Females			Bloodfed Females		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
albimanus	1.0	43.4	15.5	2.4	97.4	29.0
aztecus	6.2	84.6	38.0	12.6	108.8	45.0
quadrimaculatus	6.0	122.0	48.0	12.6	199.6	86.0

mens in their solitary confinement, we determined the exposure time needed to produce this effect.

After the mosquito had been knocked down, the DDT-slides (i, j) were removed, and one end of the box was then shut with a clean slide, whereas the other end remained covered by the bobbinet (l) on the outside of the cardboard frame. The 20 containers were then placed into a vertical position for 24 hours, so that an awakening mosquito could hang on the bobbinet and take sugar water from a cotton-wool wad. Mortality was counted after this interval of possible recovery.

The first part of the experiment, the study of irritation, was always performed from 10 A.M. to 1 P.M. or 2 P.M., the second part, *i.e.*, the observation of knockdown during the following hours, was done by repeated inspections, which, with a less susceptible species, sometimes continued for many hours.

In simultaneous control experiments with 20 mosquitoes, which were introduced in a truncate pyramid without insecticide, almost no mosquito flew off within the time needed by the 20 test mosquitoes to leave the pyramid with DDT. Twenty mosquitoes were kept, like those of the knockdown test, in solitary confinement in the same kind of rectangular boxes, but without insecticide, and under the same environmental conditions (approximate temperature 23.5° C., relative humidity about 60%). No knockdown occurred among the control mosquitoes and mortality during the following 24 hours was nil.

The minima, maxima, and means of the exposure times needed to provoke a response to irritation are given in table 1. The arithmetical means were calculated from five replicates, *i.e.*, from 100 mosquitoes altogether; the minimum and maximum values were based on the average times of the five mosquitoes which left the DDT-treated surfaces first and of the five which left last, both groups of five taken from the 100 mosquitoes tested.

The cumulative numbers of mosquitoes which left the DDT-treated surfaces are plotted against time on a logarithmic scale in graphs 1 and 2.

The arithmetical means of exposure times ESM (=*expositio stimulans minima pro medio*), on which we based our conclusions, are given in table 2 as well. Another "average" of this threshold of irritation is the *median* value or the exposure time that produces a response in 50% of the tested individuals. This median value or ES50 (=*expositio stimulans* 50) can be directly taken from the protocols, when the observations were assembled in chronological order for the 100 mosquitoes composing the five replicates; from this series of 100 recorded exposure times we read the time when mosquito No. 50 left the DDT-treated surface. The medians or ES50 values are easily read too from graphs 1 and 2.

We found that the arithmetical means and the ES50 values were roughly the same. For unfed females of *A. albimanus*, *A. aztecus* and *A. quadrimaculatus* the arithmetical means were 15.5, 38, and 48 minutes, respectively, and the ES50 values 12, 34, and 42 minutes, respectivey; for bloodfed females of the three species the arithmetical means were 29, 45, and 86, respectively, and the ES50 values 23, 35, and 87 minutes, respectively.

The minima, maxima and means of the exposure times resulting in knockdown are given in table 3. The arithmetical means were calculated from the 100 mosquitos composing five replicates, and the minima and maxima given were the average times of the five mosquitoes which had been knocked down first and those of the five last ones, respectively; both groups of five were again taken from the 100 mosquitoes tested.

The cumulative numbers of mosquitoes which were knocked down, are plotted against the exposure times on a logarithmic scale in graphs 3 and 4.

The arithmetical means of exposure times necessary to produce knockdown E″KD″M (=*expositio "knockdown" pro medio*) are also included in table 2. Concerning these exposure times, another "average" is the *median* value, *i.e.*, the exposure that produces knockdown in 50% of the

Table 2.—Comparison of averages of exposure time.

Females of *Anopheles* and Condition	Irritation			Knockdown			Relation Between Knockdown Exposure and Irritative Exposure E″KD″M:ESM
	Mean Irritative Exposure ESM (Minutes)	Interspecific Proportions	Quotient of ESM of Bloodfed to ESM of Hungry Females	Mean Exposure Resulting in Knockdown E″KD″M (Minutes)	Interspecific Proportions	Quotient of E″KD″M of Bloodfed to E″KD″M of Hungry Females	
albimanus							
unfed	15.5	1.0		87.5	1.0		5.6
			1.9			1.5	
bloodfed	29.0	1.0		128.0	1.0		4.4
aztecus							
unfed	38.0	2.4		324.0	3.7		8.5
			1.2			1.25	
bloodfed	45.0	1.5		405.0	3.2		9.0
quadrimaculatus							
unfed	48.0	3.1		254.0	2.9		5.3
			1.8			1.8	
bloodfed	86.0	3.0		461.0	3.6		5.3

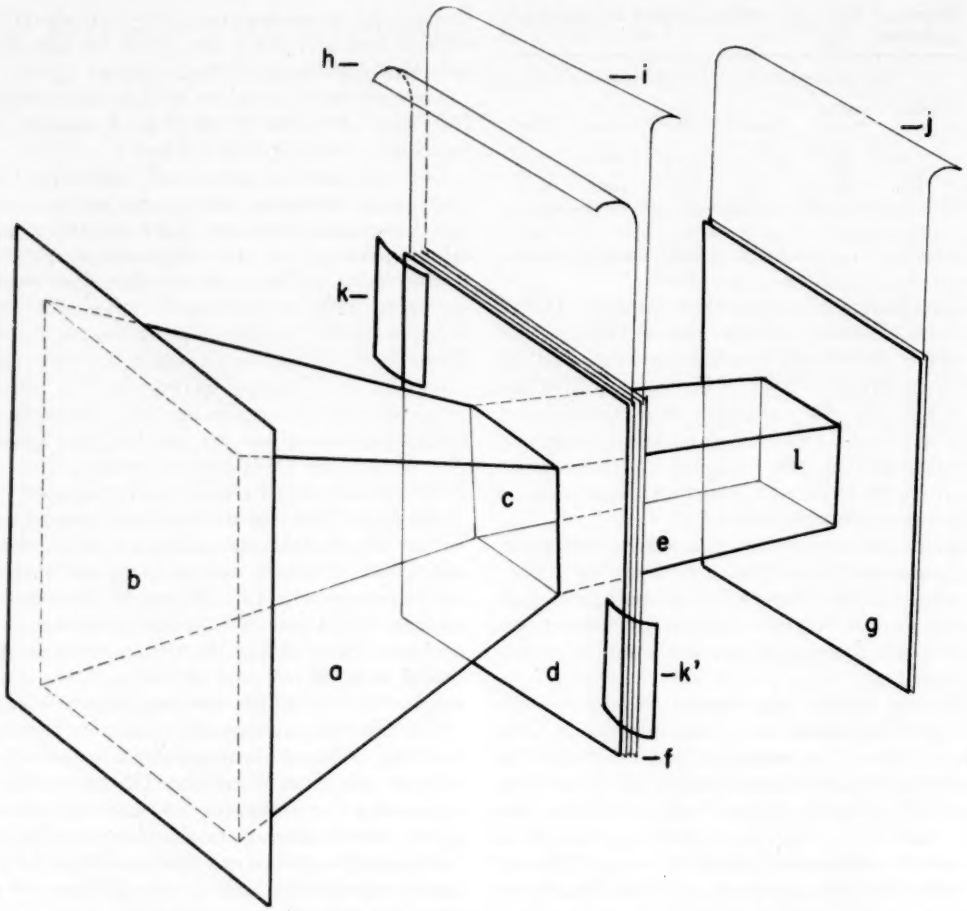


FIG. 1.—Combined device for the study of irritation and knockdown. For explanation of letters see text.

mosquitoes, which may be directly taken from the protocols or read from graphs 3 and 4. We found again, that the arithmetical means and the median values or E″KD″50 (=*expositio "knockdown" 50*) were roughly the same. For unfed females of *A. albimanus*, *A. aztecus*, and *A. quadrimaculatus* the arithmetical means were 87.5, 324, and 254 minutes, respectively, and the E″KD″50 values 82, 280, and 225, respectively; for bloodfed females of the three species the arithmetical means were 128, 405, and 461, and the E″KD″50 values 124, 400, and 470, respectively.

DISCUSSION.—When we compared the sequence of exposure times that provoked a response to irritation (listed in the unpublished data for the series of mosquitoes No. 1 to No. 20) with those of the much longer exposure times of the same series of individuals, and which result in knockdown, no correlation was found between the data recorded for the same specimen.

A mosquito which, for instance, undertakes its irritation flight after an extremely short exposure, may need a relatively short or a relatively long exposure to be knocked down; likewise, a mosquito undertaking its irritation flight as one of the last of the 20 mosquitoes may need a relatively short or relatively long time of contact with the DDT-paper in order to be knocked down.

Even when we formed five groups of the data recorded on the 100 mosquitoes, group I with the 20 that left the DDT surface first, group II with the 20 mosquitoes that left next, and so on, and when we compared the mean irritation exposure time and the average knockdown exposure for each of the five groups, no relationship was observed between these two means.

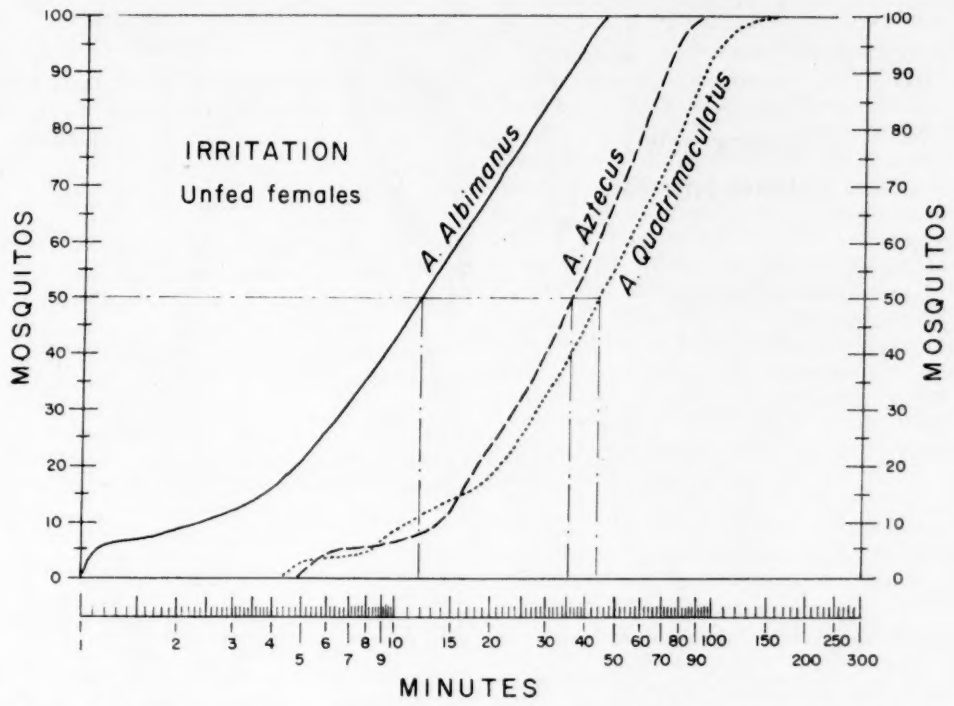
Summarizing all the observations that were made with unfed or bloodfed females of each species, the averages of exposure times were obtained, and from these and some other data presented in table 2, the following conclusions may be drawn:

I.—*Respecting Irritation.*

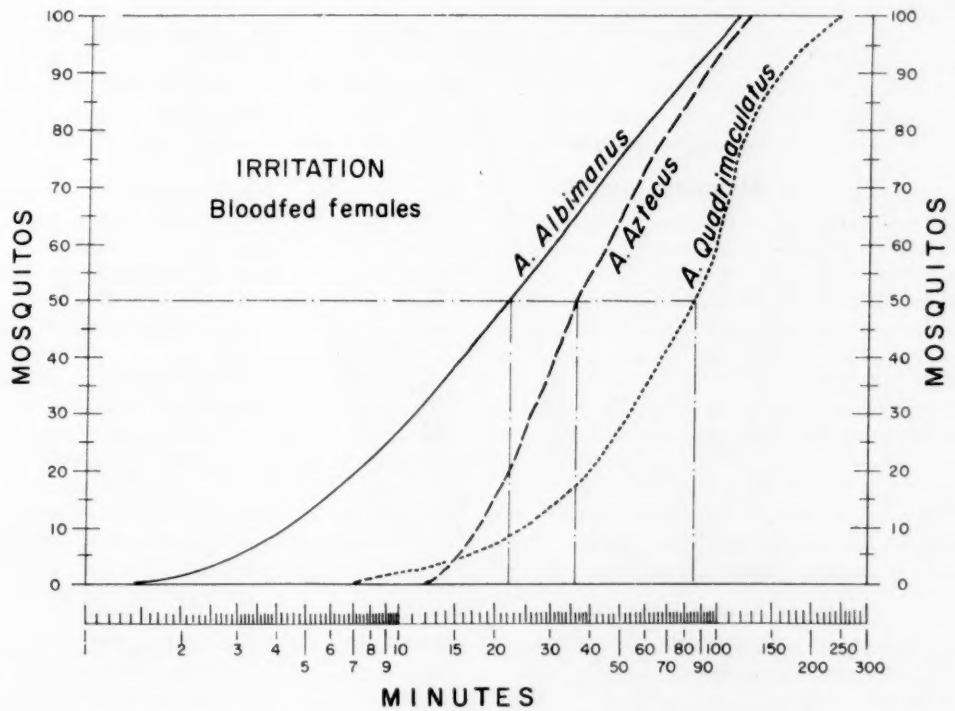
1.—Unfed females of *A. albimanus* were more irritable than those of *A. aztecus* and those of *A. quadrimaculatus*

Table 3.—Exposure time in minutes needed to produce knockdown.

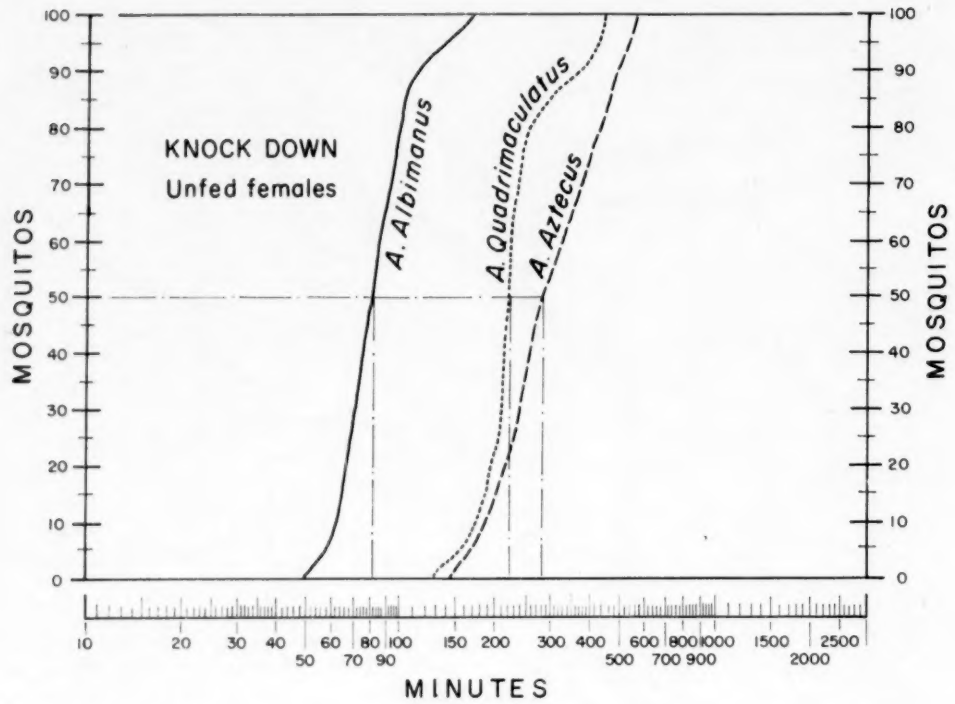
SPECIES OF *Anopheles*	UNFED FEMALES			BLOODFED FEMALES		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
albimanus	55.4	167.4	87.5	56.8	241.0	128.0
aztecus	151.2	561.0	324.0	165.2	898.6	405.0
quadrimaculatus	148.0	450.0	254.0	199.0	720.0	461.0



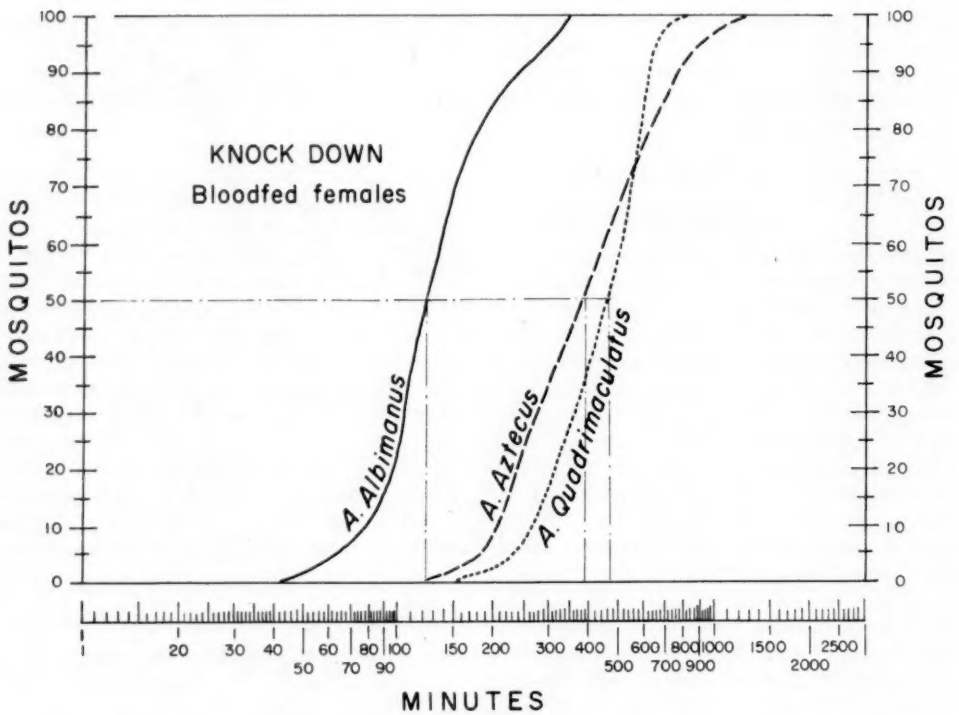
GRAPH 1.—Cumulative numbers of unfed mosquitoes which left the DDT-treated surface plotted against time.



GRAPH 2.—Cumulative numbers of bloodfed mosquitoes which left the DDT-treated surface plotted against time.



GRAPH 3.—Cumulative numbers of knocked-down unfed mosquitoes plotted against time.



GRAPH 4.—Cumulative numbers of knocked-down bloodfed mosquitoes plotted against time.

in the proportion:

$$1:\frac{1}{2.4}:\frac{1}{3.1}$$

2.—Bloodfed females of *A. albimanus* were more irritable than those of *A. aztecus* and those of *A. quadrimaculatus* in the proportion of

$$1:\frac{1}{1.5}:\frac{1}{3.0}$$

3.—The unfed females were more irritable than the bloodfed females; those of *A. albimanus* 1.9 times, those of *A. aztecus* 1.2 times, and those of *A. quadrimaculatus* 1.8 times.

Therefrom, we see that there was no significant difference with respect to the irritability of unfed and of bloodfed specimens of *A. aztecus*.

II.—*Respecting Knockdown.*

1.—Unfed females of *A. albimanus* were more susceptible than those of *A. aztecus* and of *A. quadrimaculatus* in the proportion of

$$1:\frac{1}{3.7}:\frac{1}{2.9}$$

(*A. quadrimaculatus* being more susceptible than *A. aztecus*).

2.—Bloodfed females of *A. albimanus* were more susceptible than those of *A. aztecus* and of *A. quadrimaculatus* in the proportion of

$$1:\frac{1}{3.2}:\frac{1}{3.6}$$

(*A. quadrimaculatus* being only slightly less susceptible than *A. aztecus*).

3.—The unfed females are more susceptible than the bloodfed females, those of *A. albimanus* 1.5 times, those of *A. aztecus* 1.25 times, and those of *A. quadrimaculatus* 1.8 times.

III.—*Respecting the Relation Between Irritability and Susceptibility to the Knockdown Effect.*—Though for a single specimen tested within a group no relationship existed between the degree of its irritability and of its susceptibility, the collective results indicated that the most irritant species, *A. albimanus*, was also the most susceptible one.

For knockdown *A. aztecus* needed contact with the insecticide almost nine times longer than for irritation and flight response, whereas the two other species needed only approximately five times longer exposure periods for knockdown than for irritation.

IV.—*Respecting the Relation Between Knockdown and Mortality.*—Mortality counts 24 hours after knockdown demonstrated that there was no recovery from knockdown among unfed *A. albimanus* and among unfed and bloodfed females of *A. quadrimaculatus*, and that recovery of knocked down mosquitoes in the other groups was not more than 12% to 15%. We, therefore, assume that the results on the exposure necessary to bring forth knockdown may generally be taken as an approximate measure of susceptibility to the lethal effect. An accurate estimation of susceptibility cannot be made based on the data obtained in our experiments, because it remains unknown whether some mosquitoes had taken up a lethal dose of insecticide before knockdown.

COMPARISON BETWEEN THE IRRITATION THROUGH ALMOST CONTINUAL CONTACT WITH DDT AND THE THRESHOLD OF IRRITATION PRODUCING A SINGLE RESPONSE.—The thresholds of irritation ESM, observed by means of the above described experiments and which caused a single response, cannot directly be compared with the values of the *dosis pro medio minima irritativa* (*DMI*), which were presented in the earlier paper. These values of the DMI were based, contrary to the recent observations of *one* single irritation flight, on the number of frequent flights during a prolonged period of exposure to the insecticide. Notwithstanding this dissimilarity, we find a certain parallelism between the results of the earlier work and the thresholds observed in the present investigation. It may be concluded, therefore, that in principle both methods may be used for the assessment of the gradual differences in the irritability of various species.

RESULTS OF OTHER AUTHORS AND THE NEED FOR STANDARD METHODS.—Coluzzi (1958) reported on the irritability of *A. maculipennis* Meig., *A. atroparvus* Van Thiel, *A. labranchiae* Falleroni, *A. stephensi* Liston, and *A. gambiae* Giles at contact with DDT covered surfaces. Although he, like ourselves, measured the exposure time, in minutes, necessary for the first take-off, and though his medium values (p.v.m. = "primo volo medio") are analogous to our ESM values, our results cannot readily be compared with the values he obtained because our experiments differ too much from his with regard to technical details. Nevertheless it seems to us that the majority of the species Coluzzi studied, were more irritable than the three Mexican species we dealt with.

The data Brown (1958) reported concerning the irritability of *A. albimanus* in Panamá can be compared with our values only with certain reservations.

De Zulueta (1959) performed various irritability tests with *A. sacharovi* Favr, *A. maculipennis* (*typicus*), and *A. atroparvus* of several places in Greece, Rumania, and Italy unknown to us until after concluding our study. He did not use individual mosquitoes and measure the time until their first and only take-off for flight, but released batches of five mosquitoes into a small conical exposure chamber made of transparent crystal acetate and counted the total number of take-offs during a 15-minute period, thus using a method similar to the one we did (Hecht *et al.* 1958, Barrera *et al.* 1959) when we used large transparent plastic cylinders with their circular ends covered with DDT-impregnated papers.

We believe, however, that the use of a relatively small cone is not well suited for comparative studies of irritability, because de Zulueta noticed that the mosquitoes in the control cones (put against a sheet of paper without insecticide) take off for flight much too frequently; the numbers of flights that were counted in these controls amounting to about 50% of the flights made by the mosquitoes in the cones applied to a 2% DDT-paper, and to about 40% of these undertaken in the cones of a 4% DDT-paper.

A true comparison of the irritability of different species and of local or regional populations will only be possi-

ble when the same techniques in the laboratory, as well as in the field, are used. Many studies are still needed to develop an adequate method.

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Grasshopper Damage on Short-Grass Rangeland of the San Carlos Apache Indian Reservation, Arizona[1,2]

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ABSTRACT

Over a 3-year period there was great variation in vegetative ground cover, and stands of perennial grass were more uniform and stable than those of annual grass or forbs. Blue grama and alfilaria were dominant, and perennial grasses were the most valuable and dependable forage plants. Important grasshoppers were the migratory grasshopper (*Melanoplus bilituratus* (Walker)), *Aulocara elliotti* (Thos.), and *M. cuneatus* (Gurney). All the species fed on a wide variety of grasses and forbs, but *bilituratus* and *cuneatus* showed a greater preference for forbs than did *elliotti*. Grasshopper populations ranged from 9 to 27 per square yard. Average damage was 23% to perennial grass, 26% to forbs, and 30% to annual grass. Total damage and unit-area damage per grasshopper were greatest in July, and unit-area damage per grasshopper was greater to annual grass and forbs than to perennial grass. Damage to perennial grass was greatest where the vegetation was sparse and the grasshopper population high, while the highest unit-area damage per grasshopper occurred where the vegetation was sparse and the grasshopper population moderate to light. The perennial grass ground cover was highest where *elliotti* was dominant.

The range grasshopper problem in Arizona is usually associated with open, short-grass rangeland at elevations of 4,000 to 8,000 feet. Annual precipitation varies from about 11 to 25 inches, with the snow line sometimes as low as 5,000 feet. Heaviest rains occur in July and August, with a lighter, but general, precipitation from December through March. The effect of rainfall on grasshopper populations is especially noticeable on nonirrigated land in the semi-arid areas. On poor or overgrazed rangeland, populations are greater after years with normal or above-normal precipitation than after those with unusually light precipitation. Because of its limited rainfall, Arizona is on the western border of the preferred grasshopper environment, although outbreaks occur in some areas of the State (Parker 1954).

Although there is a scarcity of published material on interrelationships of range vegetation, grasshoppers, and damage, helpful information obtained from observations on ecology of grasshoppers has been reported by Anderson & Wright (1952), Ball (1936, 1937), Ball *et al.* (1942), Barnes (1955), Cantrall (1943), Davis (1949), Isley (1938, 1946), Pfadt (1949a, 1949b), Shotwell (1930, 1938), and Wakeland (1958). This paper reports the results of studies begun in 1953 on such relationships on short-grass rangeland of the San Carlos Apache Indian Reservation.

DESCRIPTION OF STUDY AREA.—The reservation covers about 1,600,000 acres in Pinal, Gila, and Graham Counties in east-central Arizona. Most of the studies were made on Ash Creek Flat at 11 stations in sub-areas of sparse short-grass rangeland with a long history of intermittent grasshopper outbreaks (fig. 1). These sub-areas, at an elevation of about 5,000 feet, were in the east-central part of the Ash Creek Cattle Association, a 424,877-acre tract near the center of the reservation, and in portions of the Registered Herd Cattle Association, consisting of 89,340 acres southeast of the Ash Creek Association. Each station covered about 20 acres.

The plant association was mainly short grass and annual forbs. Table 1 shows the percentages of grasses and forbs in the ground cover and occurrence of plant species as indicated by surveys made in 1954 and 1955. The density and composition of the plant cover varied widely between stations and seasons, depending largely on weather conditions, soil type, topography, grasshopper damage, and extent of livestock grazing. Forbs were more variable than grasses in both composition and density of stand. Short-grass stands were generally dominated by short-lived annual plants in spring and late summer, when rainfall was favorable. Perennial grasses in the order of dominance were blue grama, curly mesquite, vine-mesquite, tobosa, desert saltgrass, three-awn, and side-oats grama. Annual grasses were little barley, fescue, stinkgrass, red sprangletop, and 6-weeks grama. Predominant annual forbs were alfilaria, *Microsteris gracilis*, tansy-mustard, sunflower, Indian wheat, lupine, and gold-fields. Perennial forbs were wild-buckwheat, wolfberry, wafer-

[1] Accepted for publication February 15, 1960.

[2] In cooperation with the Bureau of Indian Affairs, U. S. Department of the Interior.

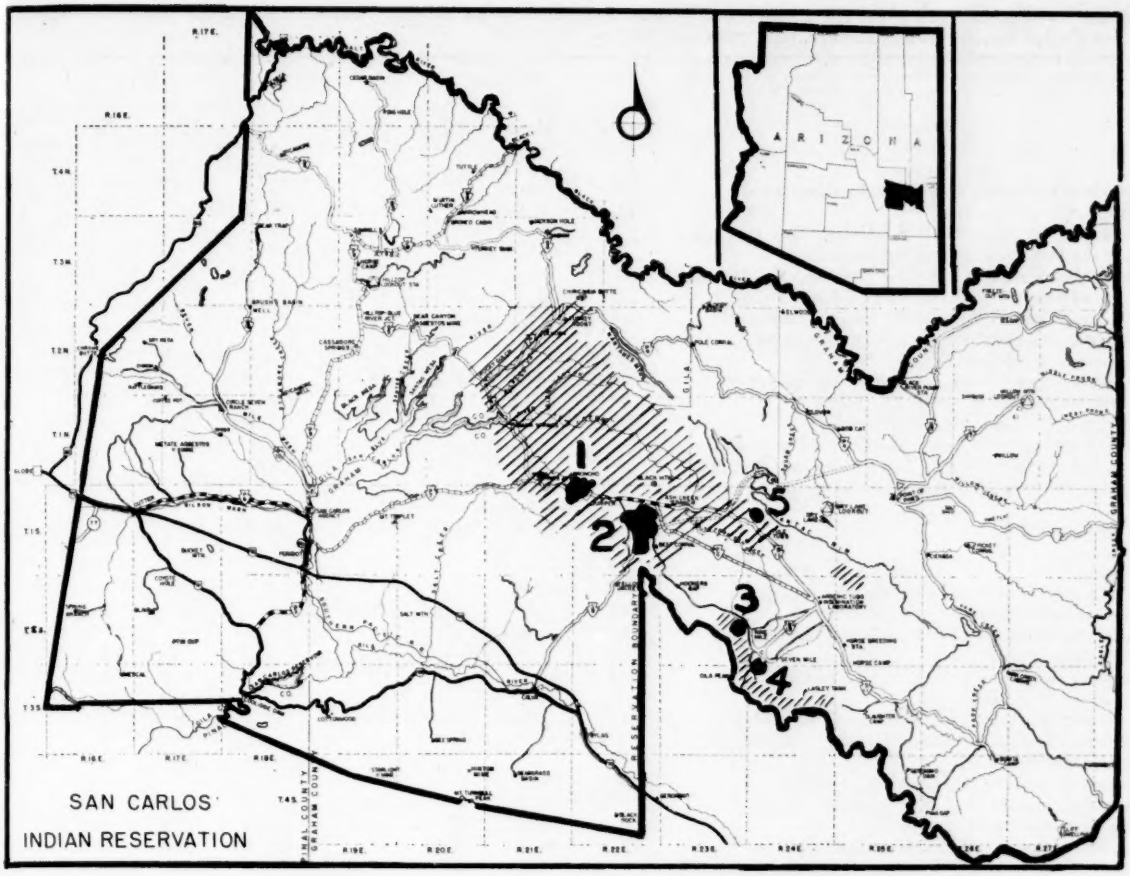

FIG. 1.—Map of San Carlos Apache Indian Reservation. Shading indicates the grasshopper-infested area. Numbers refer to subareas.

parsnip, mountain dandelion, and mariposa.

GRASSHOPPERS IN AREA.—At least 50 species of grasshoppers were present in the area. The dominant species were *Aulocara elliotti* (Thos.), the migratory grasshopper (*Melanoplus bilituratus* (Walker)), *M. cuneatus* (Gurney), and *Hadrotettix trifasciatus* (Say). All these species hatch between late March and late May. Nymphal growth required about 50 days, and adults began to appear in mid-May. Eggs were laid during the summer, and some of the adults survived until early September. There was a small second generation of *bilituratus*, which hatched in late July or during August. Hatching depended on rainfall and did not begin until the top soil was well moistened. Nymphal development of this generation required about 35 days, and adults were present from early September until frost in October or November.

FOOD PLANTS.—The plants fed upon by nymphs and adults of the dominant grasshopper species are shown in table 2. Each species did some feeding on grasses, forbs, and plant litter on the soil surface. The food-plant range of *bilituratus* and *cuneatus* was wide, including 5 grasses and 27 forbs. *A. elliotti* fed more on grasses and less on forbs, but its range of plants included 6 grasses and 17 forbs. *H. trifasciatus* preferred sparsely vegetated habitats and was observed feeding on only six species of forbs, but it also fed on the six grasses. With forbs the food-plant range of nymphs was greater than that of adults. The adults were more mobile and probably contacted preferred food plants more readily. The food-plant range of *bilituratus* was increased by feeding of the second generation late in the summer and early in the fall. The number of plant species available for grasshopper feeding was affected by weather, particularly rainfall.

SAMPLING TECHNIQUES.—Grasshopper populations at each station were estimated at 2-week intervals by the pointer method (Barnes *et al.* 1953). Over a small sampling area a few grasshoppers were stirred with a pole and counted as they moved off. The area was then examined for undisturbed grasshoppers. Grasshoppers moving onto the sampling area were not counted. At least 20 equally spaced areas were sampled at each station. The size of the area ranged from $\frac{1}{2}$ square foot for high populations to 1 square yard for low populations. The average population was recorded on a square-yard basis.

The species and their relative abundance were determined from biweekly sweep-net collections of 100 to 300 grasshoppers at each station.

Vegetation surveys were made in May, July, and September to determine the composition, ground cover, seasonal succession, and growth changes of dominant plants. Forage damage by known numbers of grasshoppers, as distinguished from that by livestock, was also recorded.

In 1953 vegetation and grasshopper damage readings were made at each station by an adaptation of the frame-

Table 1.—Percentages of different species of grasses and forbs in the ground cover at observation stations on rangeland on San Carlos Apache Indian Reservation, 1954 and 1955.

Species[a]	1954				1955			
	April	May	July	Sept.	April	May	July	Sept.
Grasses								
Aristida adscensionis L. (six-weeks three-awn)	—	—	—	—	—	—	—	0.7
Aristida spp. (three-awn)	0.3	0.3	—	—	0.4	—	0.2	0.2
Bouteloua aristidoides (H.B.K.) Griseb. (needle grama)	—	—	—	0.4	—	—	—	1.5
Bouteloua barbata Lag. (six-weeks grama)	—	—	—	—	—	—	—	1.0
Bouteloua curtipendula (Michx.) Torr. (side-oats grama)	—	—	—	0.4	—	—	—	0.4
Bouteloua gracilis (H.B.K.) Lag. (blue grama)	12.2	14.4	21.4	21.5	17.5	14.5	16.1	23.0
Distichlis stricta (Torr.) Rydb. (desert saltgrass)	—	—	0.3	—	—	—	—	0.9
Eragrostis cilianensis (All.) Lutati (stinkgrass)	—	—	—	2.6	—	—	—	20.0
Festuca pacifica Piper (fescue)	6.6	—	—	—	0.7	0.1	—	—
Hilaria belangeri (Steud.) Nash (curly mesquite)	4.9	5.6	1.6	2.2	4.8	2.4	3.3	3.7
Hilaria mutica (Buckl.) Benth. (tobosa)	—	—	0.6	0.7	0.4	0.3	0.4	0.6
Hordeum pusillum Nutt. (little barley)	0.7	1.9	—	—	1.8	0.1	—	—
Leptochloa filiformis (Lam.) Beauv. (red sprangletop)	—	—	—	1.5	—	—	—	4.6
Munroa squarrosa (Nutt.) Torr. (false buffalo grass)	—	—	—	—	—	—	—	0.3
Panicum obtusum H.B.K. (vine-mesquite)	1.1	—	0.7	0.4	—	0.3	0.1	0.7
Total grass cover	25.8	22.2	24.6	29.7	25.6	17.7	20.1	57.6
Forbs								
Agoseris spp. (mountain dandelion)	—	—	—	—	0.7	—	—	—
Ambrosia artemisiifolia L. (ragweed)	—	—	0.3	1.1	—	0.1	0.2	0.5
Amsinckia intermedia Fisch. & Mey. (fiddle-neck)	0.7	0.9	—	—	—	—	—	—
Aplopappus gracilis (Nutt.) Gray	—	—	—	0.7	—	—	0.3	0.2
Argemone platyceras Link & Otto (prickle-poppy)	—	—	—	0.4	—	0.1	0.1	—
Aster hirtifolius Blake (aster)	—	0.3	—	—	—	0.3	0.6	0.4
Baeria chrysostoma Fisch. & Mey. (gold-fields)	1.1	0.9	—	—	0.4	—	—	—
Calochortus ambiguus (Jones) Ownbey (mariposa)	0.3	—	—	—	0.4	—	—	—
Collomia linearis Nutt.	—	—	—	—	0.4	—	—	—
Cymopterus fendleri Gray (wafer-parsnip)	0.3	—	—	—	0.7	—	—	—
Descurainia sophia (L.) Webb (tansy-mustard)	4.5	5.0	—	—	3.6	0.7	—	—
Eriogonum spp. (wild-buckwheat)	0.3	0.9	2.3	2.3	0.4	0.5	1.5	1.2
Erodium cicutarium (L.) L'Her. (alfilaria)	27.0	24.0	2.3	13.7	12.1	3.8	0.4	4.3
Gutierrezia californica (DC.) Torr. & Gray (snake-weed)	—	0.3	0.3	0.4	0.4	—	0.1	—
Helianthus annuus L. (sunflower)	—	—	2.9	3.7	0.4	0.3	0.7	0.5
Hoffmanseggia densiflora Benth. (hog-potato)	—	—	—	—	—	—	0.6	0.3
Kuhnia rosmarinifolia Vent	—	—	—	0.7	—	—	—	0.1
Layia glandulosa (Hook.) Hook. & Arn.	—	—	—	—	0.4	—	—	—
Lotus humistratus Greene (deer vetch)	1.7	3.4	—	—	—	—	—	—
Lupinus spp. (lupine)	1.1	1.2	—	—	0.7	—	—	—
Lycium pallidum Miers (wolfberry)	—	—	0.7	0.7	0.4	0.2	0.5	0.2
Microsteris gracilis (Hook.) Greene	11.9	7.2	1.0	—	8.4	—	—	—
Plantago purshii Roem. & Schult. (Indian wheat)	—	2.8	—	—	—	0.1	—	—
Sphaeralcea spp. (globe-mallow)	—	0.6	0.7	0.4	—	0.2	0.3	0.7
Trifolium spp. (clover)	0.3	0.9	—	—	—	—	—	—
Undetermined species	—	0.3	0.6	—	—	—	—	2.6
Total forbs cover	49.2	48.7	11.1	24.1	29.4	6.3	5.3	11.0
Total grass and forb cover	75.0	70.9	35.7	53.8	55.0	24.0	25.4	68.6

[a] Charles T. Mason, University of Arizona, Tucson, identified the plants.

point sampling method described by Levy & Madden (1933). A wooden horizontal bar $\frac{5}{8}'' \times 1\frac{1}{4}'' \times 24''$ had vertical steel supports made of strips $\frac{1}{16}'' \times \frac{5}{8}'' \times 14\frac{1}{2}''$ bolted to each end. The steel supports were pointed at the lower end so that they could be driven or pushed into the ground about $2\frac{1}{2}$ inches. Ten steel needles $\frac{3}{32}$ inch in diameter and 12 inches long were inserted through holes at 2-inch intervals in the horizontal bar with the first and last holes 3 inches away from the vertical end supports. The needles were pushed downward until the points struck plants, litter, or bare soil. All plants touched by a descending needle were recorded. Sometimes, especially in dense vegetation, more than one species was hit by a single needle. Two readings were made with the frame-point sampler at each station. The sampler was placed against a permanent stake $\frac{1}{2}$ inch thick by 2 inches wide so that the needles were at a right angle to the wider surface. This operation was repeated on the opposite side of the stake, making 20 individual needle observations at each station. By periodic sampling throughout the season it was possible to measure the vegetation as it matured or was damaged by grasshoppers. The relative ground cover of different plant types was estimated by visual inspection.

In 1954 a step-point method of sampling was used. Twenty or 30 sampling points were equally spaced on a paced line transect 1,000 or 1,500 feet long at each station. They were determined by counting the paces be-

Table 2.—Plants fed upon by four grasshopper species on rangeland on San Carlos Apache Indian Reservation, 1953–55.[a]

PLANT SPECIES	*Melanoplus bilituratus*	*Melanoplus cuneatus*	*Aulocara elliotti*	*Hadrotettix trifasciatus*
Forbs				
Agoseris glauca	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 3, 4, 5, A	
Amsinckia intermedia	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 3, 4, 5, A	
Aplopappus gracilis	A	A		
Argemone platyceras	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	1, 2, 3	1, 2, 3
Aster hirtifolius	4, 5, A	4, 5, A		
Baccharis pterontoides	5, A	5, A		
Baeria chrysostoma	1, 2, 3	1, 2, 3	1, 2, 3, 4	
Calochortus ambiguus	2, 3	2, 3	1, 2	
Collomia linearis	1, 2, 3	1, 2, 3	1, 2, 3	
Cirsium ochrocentrum	4, 5, A	4, 5, A		
Cymopterus fendleri	2, 3	2, 3	1, 2, 3, 4	
Descurainia sophia	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	1, 2, 3, 4	
Eriogonum spp.	4, 5, A	4, 5, A		
Erodium cicutarium	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	2, 3, 4, 5, A
Gilia spp.	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	
Gutierrezia californica	4, 5, A	4, 5, A		
Helianthus annuus	3, 4, 5, A	3, 4, 5, A		A
Kuhnia rosmarinifolia	5, A	5, A		
Layia glandulosa	1, 2, 3, 4, 5	1, 2, 3, 4, 5	1, 2, 3, 4, 5	2, 3, 4
Lotus humistratus	1, 2, 3, 4, 5	1, 2, 3, 4, 5	3, 4, 5	2, 3, 4
Lycium pallidum	A	A		
Microsteris gracilis	1, 2, 3, 4	1, 2, 3, 4	1, 2, 3, 4	
Oenothera flava	1, 2, 3, 4, 5	1, 2, 3, 4, 5	2, 3, A	
Plantago purshii	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	2, 3, 4, 5, A	2, 3, 4
Platystemon californicus	1, 2, 3	1, 2, 3		
Sphaeralcea emoryi	4, 5, A	4, 5, A	A	
Trifolium spp.	1, 2, 3, 4	1, 2, 3, 4	2, 3, 4	
Grasses				
Bouteloua gracilis	3, 4, 5, A	4, 5, A	2, 3, 4, 5, A	4, 5, A
Distichlis stricta			A	3, 4, 5, A
Eragrostis cilianensis	1, 2, 3, 4, 5, A	A	A	4, 5, A
Hilaria belangeri	4, 5, A	4, 5, A	2, 3, 4, 5, A	3, 4, 5, A
Hordeum pusillum	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	1, 2, 3, 4, 5, A	4, 5, A
Leptochloa filiformis	1, 2, 3, 4, 5, A	A	A	4, 5, A
Plant Litter(on soil)				
Various species	3, 4, 5, A	3, 4, 5, A	3, 4, 5, A	3, 4, 5, A

[a] Figures 1 to 5 represent the respective nymphal instars; the letter A indicates adults.

tween points. At each point a record was made of the plants included in a notched space 1-inch long and $\frac{1}{4}$-inch deep on the sole of the right boot toe. Brown (1954) mentions use of the toe cap of a boot to determine the sampling point.

In the spring of 1955 a combination of a quadrant technique with the step-point method was used. A square-foot wire frame was placed on the ground at the toe point, and the ground cover of the plant types within this quadrant was estimated. The plant-composition readings were taken from the $\frac{1}{4}'' \times 1''$ toe-point notches, as in 1954. (Both a step-point and a frame-point-step-point method of sampling vegetation were described by Evans & Love (1957).) The frame-point sampler (used in 1953) was used again in 1955 for summer and fall vegetation surveys in which 20 to 30 stops involving 200 to 300 individual needle-point readings were made along a 1,000- to 1,500-foot transect line at each station.

Measurements of short grass were based on the blade length. Herbaceous or weedy plants were measured by the basal leaf spread and shrubs by their crown spread. The percentage of grass and forb ground cover was obtained by dividing the number of needles that touched grasses or forbs by the total number used. The number of needle contacts with a plant species was used to determine its occurrence in the total plant cover.

In 1953 grasshopper-damage readings were taken on all plants touched by needle points as well as on plants selected at random. In 1954 readings were made on plants included in the right boot sole notch. In 1955 the method was similar to that used in 1954, except that the number of stops was reduced to 10 or 15 per station, and readings were also made at each stop on the plants hit by the first descending needle of the frame-point sampler. Again 20 or 30 units were sampled at each station. If the ground cover was 100%, there would be 20 or 30 unit readings of grasshopper damage at each station, but usually the cover was less than 100%.

Damage was estimated from observations on the foliage and other parts of plants. In 1953 observations on the same plants in spring, summer, and fall showed that the greatest damage was on blades of short grass, and occurred after annual plants had matured. From 25 to 50 blades and all stems were examined at each point where contact was made with perennial grass. Culms were broken off and placed in a cardboard box having several ruled lines on the baseboard. All blades were counted and those damaged recorded. The amount of damage was calculated by estimating the foliage consumed. The length of damaged blades was estimated by comparing their width with that of nearby undamaged blades of the same species. The length of a destroyed blade was estimated by observing its position on the culm.

Damage to flowering stems was determined as the per-

Table 3.—Grasshopper damage to range vegetation on San Carlos Apache Indian Reservation, 1953–55.

Month or Year	No. of Observations[a]	Ground Cover (%)	No. of Grasshoppers Square Yard[b]	Grasshopper Damage	
				Total %	Per Grasshopper per Square Yard[c] (%)
			Perennial grasses		
May	14	26	18.6	16.4	0.62
July	15	24	16.4	37.9	2.28
September	15	28	12.7	15.3	1.01
Average	—	—	—	—	1.32
1953	15	24	11.2	18.2	1.38
1954	15	25	20.2	32.4	1.58
1955	14	29	16.2	19.2	.97
Average	—	—	—	—	1.32
			Annual grasses		
May	5	5	27.3	53.3	2.39
July	2	3	15.2	62.6	4.27
September	8	16	10.6	8.0	.75
Average	—	—	—	—	1.77
1953	4	6	16.1	29.8	1.99
1954	7	5	17.9	32.8	2.08
1955	4	27	15.5	26.7	1.01
Average	—	—	—	—	1.77
			Dominant forbs		
May	13	28	15.4	16.2	1.10
July	11	11	14.8	43.1	3.09
September	12	11	13.1	20.6	1.23
Average	—	—	—	—	1.75
1953	10	22	9.1	27.7	2.64
1954	15	19	20.2	35.2	2.05
1955	11	10	11.5	11.6	.52
Average	—	—	—	—	1.75

[a] Refers to sampling of subareas not individual stations.

[b] Computed as the average number over a seasonal period, as from the time of hatching to May, to July, or to September, and the three seasonal cumulative average populations were added and divided by 3 to obtain the mean effective annual population for the hatching-to-May, hatching-to-July, and hatching to-September periods.

[c] Average of individual computations for each subarea observation.

centage of stems cut off or girdled.

The damage on forbs was estimated in relation to the entire plant or the herbage of that plant included in the sampling unit. Leaves of browse shrubs were generally examined in units of 25.

RESULTS.—Table 3 shows grasshopper damage in the five representative subareas during the period 1953–55. Some of the subareas had two or more stations, but results in such subareas were combined.

The total damage caused by grasshopper populations of 9 to 27 per square yard ranged from 8% to 63%. A threatening to severe infestation in the spring of 1955 was reduced to noneconomic numbers by an application of aldrin spray in late May and early June, and the population remained low for the rest of the grasshopper season. Perennial grasses were more widely distributed, denser, and more uniform of stand than were annual grasses or forbs. Forbs comprised a larger percentage of ground cover than annual grasses. The highest grasshopper populations were recorded in May, when most of them were in the nymphal stage, but the damage to vegetation was greatest in July after the grasshoppers had matured and fed for a longer period on vegetation that was declining in succulence and density because of dry weather. Other factors being equal, the damage usually increased with an increase in the population or a decrease in the vegetative ground cover. Populations and damage were highest in 1954, a year in which populations were building up and no control was attempted. The damage per grasshopper per square yard was higher in July than in May or September, and higher on forbs and annual grasses than on perennial grasses.

Table 4 shows the relationship between the blade length of perennial grasses and grasshopper damage. Observations were made in May, July, and September in five subareas in 1953 and 1954 and in four subareas in May 1955. One of the subareas had no perennial grass in May 1955 and data from the July and September observations in 1955 for all five of the subareas were excluded from consideration because the grasshopper population was controlled by spray application in late May and early June. The number of pertinent observations was thus reduced from 45 to 34. As the length of the grass blades increased there was an increase in the perennial

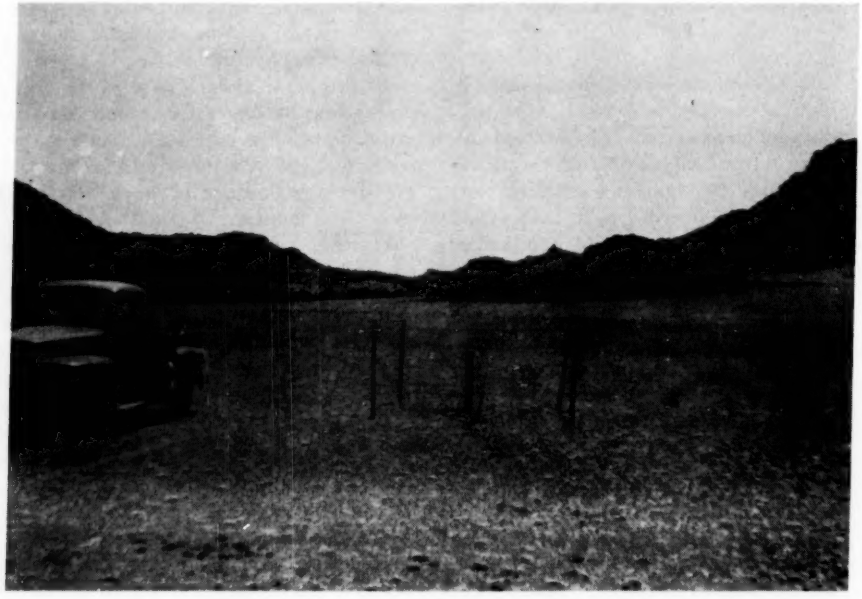

FIG. 2.—Short-grass stand severely damaged by grasshopper feeding at observation station 10, subarea 4, June 1954. By late summer the perennial grass was completely destroyed and the sod dead.

FIG. 3.—Scene in June 1959 showing recovery of vegetation at observation station 10, subarea 4.

Table 4.—Relation between blade length of perennial grasses, density of vegetation, grasshopper populations, and damage, 1953–55, 34 observations.

Blade Length (Inches)	Percentage of Vegetative Ground Cover				Number of Grasshoppers per Square Yard	Observations	Damage per Grasshopper per Square Yard (%)
	Perennial Grasses	Annual Grasses	Forbs	Total			
0–0.9	15.9	1.8	12.8	30.6	31.6	9	2.15
1–1.9	24.4	3.7	20.6	48.7	13.0	13	1.35
2–2.9	30.0	2.5	16.2	48.7	13.2	7	1.16
3–4.6	37.8	3.0	9.2	50.0	7.5	5	.78
						Weighted average	1.44

grass cover, a tendency toward an increase in the total vegetative cover, a decrease in the grasshopper population, and a decided decrease in the percentage of damage to perennial grass per grasshopper per square yard. There was no uniform or progressive relationship between perennial grass blade length and the percentage of vegetative ground cover by annual grass or forbs.

The effect of grass volume, determined chiefly by stand density and blade length, and grasshopper populations on damage per grasshopper per square yard is shown in table 5 for several representative observation sites. Perennial-grass damage per unit area apparently depends largely on the volume of the grass, the number of grasshoppers, the food preferences of different species, and the availability of other food plants. The greatest damage occurred at sites 1 and 2, where the stand of vegetation including perennial grass was relatively light to moderate, the blade length short to moderate, and the grasshopper population moderate and comprised mainly of *elliotti*. The damage was lower at sites 3 and 4, where stands of grass and other plants were sparse, the grass blades short and the grasshopper populations moderate, consisting mainly of *bilituratus* and *cuneatus*. The low percentage at site 5 was caused by a very high population that almost destroyed the sparse stand of short vegetation and there was only a small portion of the total grass available for each grasshopper. The low percentages at sites 6 and 7 could probably be attributed to the moderately dense grass with fairly long blades associated with light to moderate grasshopper populations. The low damage at site 8 was probably due to the low grasshopper population in a moderate stand of grass together with the dominance of *bilituratus*, a species that usually prefers forbs over grasses.

Although all three species lived in mixed habitats and fed on perennial and annual grasses and forbs, there was evidence that *elliotti* had a greater preference for perennial grasses than *bilituratus* or *cuneatus*. In the summers of 1953 and 1954 *elliotti* was dominant at four sites where the perennial grass ground cover was 31.9%, the damage to perennial grass blades 54.3%, and the damage per grasshopper per square yard 3.3%, while *bilituratus* and *cuneatus* were dominant at six sites and the respective percentages were 20.4, 38, and 2.16. In 35 samplings at

Table 5.—Effect of perennial grass density and blade length and grasshopper populations on summer damage to perennial grass blades at individual sites, July 1953 and 1954.

Site No.	Maximum Blade Length (Inches)	Per Cent Ground Cover by—		Grasshoppers		Damage per Grasshopper per Square Yard (%)
		All Plants	Perennial Grasses	No. per Square Yard	Dominant Species	
1	0.9	33.9	21.8	14.6	*elliotti*	4.79
2	1.6	31.2	16.0	11.9	*elliotti*	4.14
3	.7	18.0	10.0	16.8	*bilituratus* and *cuneatus*	2.98
4	1.0	21.1	16.2	18.4	*bilituratus*	2.08
5	.2	15.0	10.0	52.5	*elliotti*	1.88
6	3.1	50.0	35.0	7.2	*elliotti*	1.83
7	2.2	55.0	45.0	20.6	*elliotti*	1.15
8	1.2	28.8	20.1	6.1	*bilituratus*	1.25

rangeland sites between May 1953 and May 1955, the perennial grass ground cover averaged 18.8% in 17 of the samplings where *bilituratus* and *cuneatus* were dominant and 29.4% in 18 samplings where *elliotti* was dominant.

Very heavy populations were highly destructive to perennial grass as well as to annual grass and forbs. Figure 2 shows a location where during the summer and fall of 1954 a mixed population of 42 to 64 per square yard destroyed more than 99% of all vegetation. The vegetative ground cover was 45% at the location early in May. Figure 3 shows recovery of the vegetation following grasshopper control in 1955 and controlled grazing during the period 1955–59.

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Infestation of the Imported Fire Ant, *Solenopsis saevissima* v. *richteri*, at Fort Benning, Georgia[1]

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ABSTRACT

In the fall of 1956 an infestation of the imported fire ant began at Fort Benning, Georgia, and became a serious problem the next year. Approximately 300 cases, five of which were considered to have been serious, were treated for fire ant stings at the United States Army Hospital. By the end of the summer of 1957 injuries had dropped to very few cases owing to a successful control program and full cooperation of the post personnel.

While chemical control measures were being applied, an educational program was initiated in order to acquaint the personnel of the post with measures to prevent injuries, and first aid procedures to follow in case of injury.

The pattern of invasion and dispersion indicated that the family automobile played an important role in the infestation and spread of the imported fire ant.

In late September 1956, a few imported fire ant mounds were discovered in a housing development and on an incoming highway at Fort Benning, Georgia. This was the first sign of an invasion which developed into a large infestation and a medical problem during the following year. With the coming of warm weather in 1957 the imported fire ant appeared suddenly in large numbers and inflicted many human injuries.

After establishment along the roads and in three housing areas, the fire ant followed a definite pattern, spreading to other portions of the military reservation as the summer progressed. During early summer the fire ant also appeared in nearby Columbus, Georgia, located on the Chattahoochee River, which forms the Georgia-Alabama boundary.

The early detection of the insect, the geographical arrangement of the post, and the efficient administration and cooperative spirit of the military personnel were conditions which were ideal for an investigation of this type.

MEDICAL PROBLEM.—Approximately 300 individuals were treated for ant stings at the United States Army Hospital at Fort Benning. Victims were of all ages, but the majority were small children who were attacked while

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playing in what they obviously thought were small sand piles.

A small percentage of the patients had serious hypersensitive reactions to the stings. Three children showed marked edema, cyanosis, and respiratory difficulties which could have been fatal if immediate medical attention had not been obtained. One of the three, a girl, had received stings on four previous occasions without any abnormal symptoms. Her hypersensitive reaction to the fifth attack was rapid and developed into the most serious case of the group. All children recovered rapidly and without complications. Two middle-aged officers who received only a few stings reacted violently. One received six stings on one leg which resulted in total body edema and nausea, both of which lasted for 2 days. The other received three stings on the top of one foot. Within 3 minutes he had severe hives, and fainted three times within a period of 10 minutes. He described himself as feeling "confused" during this period. He received immediate medical attention and went to work the next day. Several other individuals also showed abnormal reactions. However, one child, 1½ years of age, received 220 stings on the entire right half of his body, but showed little more than normal reaction.

The majority of the victims showed the following common symptoms: The initial sensation was a moderate sting. Within 5 minutes a raised area (vascular constriction) about 5 mm. in diameter appeared. This was followed by several hours of moderate itching. After 24 hours blisters formed, followed by pustules. Scabs usually formed about the third day, resulting, in most cases, in bad scars. Among young children severe secondary infection often resulted from scratching the wounds. In many cases, victims had large wheals (1 inch or more in diameter) around the wounds. When there were many stings on a limb or appendage, severe edema occurred locally, followed by inflammation and high temperature of the area.

The victim was usually stung within 5 to 10 seconds after the ant got on his body. Since the insects move quite rapidly, it is possible for several dozen to be on the victim before the stings are initiated. Consequently, one may be stung by many ants at a time. On one occasion the writer photographed his hand 5 seconds after it was thrust into a broken hill. The resulting picture showed 94 fire ants on the hand. He has seen many neighborhood children attacked. Young children, especially under 5 years of age, seemed to stiffen and scream without making any effort to rid themselves of the insects.

Professional treatment of the wound was directed toward eliminating hypersensitive reactions and reducing local infection. Injured individuals 13 years of age and older were given graduated dosages of pyribenzamine, while children 12 and under were given graduated dosages of benadryl. Pustules were treated like any common sore. Secondary infection seemed to have been reduced by immediately bathing the injured area before the child had the opportunity to scratch.

CONTROL AND PREVENTION OF INJURY.—All control measures were aimed toward the elimination of human injury. Efforts were divided into two programs: (1) the actual chemical control of the ant, and (2) the education of the personnel of the post.

Under the first program, chemical control began early in the spring of 1957 using "GI spray" (2% chlordane plus 5% DDT in kerosene) in a mound-to-mound treatment. Knap-sack sprayers were used to direct the insecticide into the mounds and onto the surrounding ground within an 8-foot radius of the mound. This treatment was quite ineffective. A very small percentage of the ant population was killed, the majority escaping through radiating tunnels and establishing colonies elsewhere. The gross effect of this treatment was the dispersion of the original populations and the establishment of more numerous ant hills.

During the first week of July 1957 fifty tons of 5% granulated heptachlor was purchased and a total-area treatment was begun. Using cyclone seeders, crews of five or six men, walking abreast, distributed the material at 40 pounds per acre. Two other men followed, broke open all mounds, and poured a handfull of the material therein. At first the chemical control program was concentrated around housing areas and playgrounds. Later it included parade grounds, shoulders of roads, ranges, and other outlying areas of infestation.

This treatment was an immediate success, eliminating all signs of fire ant mounds in treated areas, while in untreated areas, the ants continued to thrive. Up until the winter of 1957 no treated areas had been reinfested.

After this treatment was completed, injured persons seen at the hospital decreased from as many as 30 per day to one or two per week.

For 2 or 3 weeks after the beginning of this treatment many large insects, such as grasshoppers, cockroaches, and mole crickets were found dead on lawns. Also, about 2 dozen dead birds were found. After a month had elapsed, no more large insects or birds were found dead, and the avian population continued to thrive. The earthworm population did not appear to be affected.

The second program dealt with the education of the families within the army post. Within a week's time, by the use of daily bulletins, memoranda, and the post newspaper, all personnel were made fire ant conscious. Through these facilities the insects and their mounds were described, the serious consequences of fire ant injuries were discussed, and home first aid measures were outlined. Phone calls from distressed and interested people poured into the Preventive Medicine Office where the callers were given further information. The interest developed by the people made control operations more effective.

METHOD AND PATTERN OF DISPERSION.—The most graphic observation during this study was the importance of the automobile as means of dispersing the imported fire ant.

The large population of ants in the spring of 1957 seemed to indicate that the spring population was not entirely progeny of the small population of the preceding fall. It would seem that if the fire ant population had been at Fort Benning for several years, multiplying unseen as they sometimes do, the final outbreak would have been more uniform over the 182,779-acre reservation. This spring outbreak occurred only on the shoulders of two incoming roads and in three family housing areas. These housing areas were widely separated, forming a triangle with the apices being a mile

or more distant from each other. The units were all adjacent to lush parade grounds, fields, or vast lawns surrounding military buildings, none of which were infested although they were theoretically attractive to the insects. No outlying areas were infested at that time. There were no known infestations around the incoming railroad. Since these housing areas have been landscaped for many years, infestation by means of imported nursery stock can virtually be ruled out. One of the above-mentioned housing areas was occupied by young officers' families, one by older officers' families, and the other by noncommissioned officers' families. It may seem significant that the area occupied by the young officers (who do far more traveling than the other two groups) was more heavily infested than were the other two areas. The older officers' units were more heavily infested than the noncommissioned officers' area. A typical area considered to be highly infested was a court 2500 square yards in area, which had 38 mounds. The older officers' area averaged about two to four mounds per lawn, and the noncommissioned officers' area averaged a mound per two to four lawns.[3] Barrack areas housing recruits and other enlisted personnel were not infested at that time.

In late August 1957 the fire ants began to appear in outlying areas. In practically all these cases, the mounds were seen near parking lots of headquarters buildings, ranges, and other locations where young officers regularly park their family cars.

Several people have reported finding winged females in their automobiles. However, it is thought that the insects were chiefly transported on the undersides or outside surfaces of the vehicles since the ants will attack or crawl on any nearby object when the mound is disturbed.

In conclusion, there is some evidence that the automobile played a major part in the imported fire ant infestation of Fort Benning. The ant was probably brought to the reservation in or on the automobiles of touring personnel who went through infested areas. After the incoming roads and the housing areas were heavily populated with the insect, it was probably transported by cars to outlying areas, always beginning its infestation at or near parking lots. The automobile should be considered a major means of dispersing the imported fire ant.

[3] This estimate of population is given in lieu of a more accurate survey which could not be made at the time.

Systemic Insecticides for Control of the Introduced Pine Sawfly, *Diprion similis*, with Notes on Parasite Survival[1]

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ABSTRACT

Injection of white pines of 4- to 6-inch diameter at breast height with phorate, demeton, Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate (hydrogen oxalate salt)), and Shell 3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate) resulted in sufficient translocation into the needles to kill feeding larvae of the introduced pine sawfly, *Diprion similis* (Htg.). Chipman R-6199 remained toxic to the larvae for 2 years. Phytotoxicity was not observed in any of the injected trees. Hymenopterous and dipterous parasites caged with foliage from injected trees were not adversely affected by the systemics in the foliage.

In a review on the use of systemic insecticides, Ripper (1957) indicated that a wide variety of insect pests including leaf-feeders or defoliators could be controlled by this type of chemical. Giese *et al.* (1958) briefly reviewed some of the previous work with systemics in coniferous trees and in their studies reported 100% control of the needle-feeding larvae of the red-headed pine sawfly, *Neodiprion lecontei* (Fitch), 2 to 5 days after implantation of the trees with varying concentrations of demeton. Studies on the control of the introduced pine sawfly, *Diprion similis* (Htg.) on eastern white pine, *Pinus strobus* L., with systemic insecticides were undertaken in Wisconsin in 1958 and 1959 and supplemental data on the effects of foliage from injected trees on biological agents of control were accumulated.

MATERIALS AND METHODS.—Natural regeneration white pine trees 4 to 6 inches diameter at breast height and 20 to 30 feet high were selected for treatment. The lower tree branches were removed from all trees to a height of 5 feet primarily to prevent movement of the toxicants into branches with very few needles. Three insecticides, demeton, phorate, and Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate (hydrogen oxalate salt)), were employed in 1958 and one, Shell 3562 (dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate), in 1959. Technical grades of demeton, phorate, and Shell 3562 were used, however, R-6199 was used as a 50% aqueous solution. Each insecticide-treated tree received 8 gm. of active ingredient except for one series of trees in 1959 which received only 4 gm. (Shell 3562). Two types of insecticide application were used: (1) injection of the systemic into holes drilled at the base of the trunk or (2) banding the basal trunk area with systemic. Drilled and undrilled control trees were also included in the experiment. Each treatment was replicated three times in 1958, and in 1959, with Shell 3562, four replicates were used.

Injection consisted simply of pipetting equal portions of the dosage into each of three (1958) or four (1959) holes equally spaced around the tree trunk. The holes were $\frac{1}{2}$ inch in diameter, approximately $1\frac{1}{2}$ inches deep, and drilled at an angle of 45° to the main axis of the

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tree. These were placed from 6 to 12 inches above the soil surface. After introducing the insecticides, the holes were corked to prevent leakage, and the basal 2 feet of each tree was enclosed with chicken-wire screening to exclude mammals. Bark application was made at the same level on the trunk as injection. Cloth ground mats, 1 yard square, were placed under each tree to collect falling larvae and frass. Plastic screen sleeve cages with known numbers of immature larvae were set up on the trees (mid-crown area) 1 day before injection in 1959 so that normal feeding would be established. Mats were examined daily and the sleeve cages twice weekly. Tests of larval mortality were undertaken for both generations in 1958 and 1959 (except with Shell 3562) to provide data on insecticide persistence. Beneficial insects were caged with foliage cut from both treated and untreated trees to establish the compatability of chemical and biological control agents. Needles and twigs from the lower, mid, and upper crown levels of control and injected trees (1958) were removed at 2-week intervals throughout the season. These were quick frozen and are currently being bioassayed to provide data on the translocation and persistence of the systemic insecticides.

RESULTS AND DISCUSSION.—Preliminary data accumulated in 1958 on first generation larvae indicated that large numbers of larvae dropped from all injected trees onto the cloth mats. Larval mortality occurred more rapidly in open grown trees than in partially or wholly shaded trees. In phorate-injected trees, mortality was noticeable 2 days after injection and continued for approximately 7 days. Demeton-injected trees showed approximately the same response but on two of the trees, larval mortality was delayed for up to 6 days. Trees injected with R-6199 began killing the first generation larvae 3 to 4 days after injection and continued for approximately 6 days. Bark banding application of systemics gave little or no control. One tree, however, where demeton was used did kill large numbers of sawfly larvae. It was observed later that the application was made over the spot where a branch had been removed and this, in fact, acted as an injection hole. Daily examination of frass accumulation on the cloth mats indicated a progressive decrease under the injected trees and a progressive increase under the control trees.

Approximately 3 weeks after the initial injection, and when second generation larvae were numerous in the field, the sleeve cages were set up in the mid-crown areas of the injected, banded and control trees. Mortality in both phorate- and demeton-treated trees varied from 18% to 28% but this was comparable to the controls (23%). Consequently, it is concluded that phorate and demeton or their toxic equivalents did not persist in lethal concentrations in the caged needles. In comparison, larval mortality in the R-6199-injected trees reached 84% within approximately 2 weeks, indicating a high degree of toxicity and persistence. Mortality in the R-6199-banded trees was not significantly different from that recorded for other banded trees.

After 1 year, cages were again placed on the 1958-injected trees and on trees freshly injected with Shell 3562. No significant mortality of first generation 1959 larvae occurred in the phorate-, or demeton-injected trees or the controls (table 1). This confirmed our 1958 observations on second generation larvae. The R-6199-injected trees, however, showed a 92% larval mortality, most of which occurred in 2 to 4 days. It was practically impossible to locate naturally occurring larvae on the R-6199-injected trees in 1959 even though eggs were deposited in the needles. Trees treated with 8 gm. of Shell 3562 killed an average of 77% of first generation larvae (range 64% to 92%). Larval mortality was observed approximately 3 days after injection and most of the larvae were dead within 11 days. At 4 gm. per tree an average of 81% of the larvae succumbed (range 73.9% to 86.9%). Mortality in the control trees did not exceed 16%.

Table 1.—Mortality of *D. similis* larvae on white pine trees injected with phorate, demeton, and Chipman R-6199, on July 16, 1958, and with Shell 3562 on July 16, 1959.

Insecticide	Dosage per Tree (Gm.)	Per Cent Larval Mortality			
		1958		1959	
		1st Generation[a]	2nd Generation[b]	1st Generation[b]	2nd Generation[b]
Demeton	8	heavy	24.0	18.2	—
Phorate	8	heavy	27.7	0.0	—
Chipman R-6199	8	heavy	84.0	92.0	90.4
Shell 3562	8	—	—	77.0	100.0
	4	—	—	81.0	100.0
Control		light	26.7	11.8	0

[a] Estimated from larval drop to cloth mats (1 yd. sq.); many larvae weakened by systemics crawled from mats and were lost.
[b] Estimated from known numbers of larvae enclosed in screen sleeve cages on branches.

Second generation larvae were caged on the R-6199- and Shell 3562-injected trees during August. Larval mortality in the R-6199-injected trees was 90.4%. In the Shell 3562-injected trees, 100% mortality occurred in 48 hours on trees previously injected with 8 gm. and in 96 hours on those injected with 4 gm. active ingredient. There was no mortality in the controls.

It appears then that Chipman R-6199 or its toxic equivalent persisted in insecticidal concentrations in the white pine trees for two consecutive seasons or through four generations of the sawfly. Phorate and demeton were partially effective through only one generation and did not have the residual properties of R-6199. Shell 3562 showed considerable promise having remained effective throughout one season (1959) or two generations of the pest.

Caged adult hymenopterous and dipterous parasites were exposed to foliage cut from trees injected with the previously mentioned insecticides and control trees to test their compatability with the various systemics. Adults of the introduced pine sawfly caged with foliage from injected trees lived normal life spans (approximately 8 days) and deposited eggs in the needles with no deleterious effects. Adults of *Monodontomerus dentipes* (Dalm.), the most important biotic agent of the complex, caged in the same manner also lived normal life spans with no mortality after 4 days of exposure. Similar results with field-collected parasites in the families Sarcophagidae and Tachinidae were obtained. In no instance could any mortality of parasites or host adults be attributed to the injected material.

Phytotoxicity was never observed in the 4- to 6-inch white pines with standard dosages of 8 gm. or less, in fact, some trees given an additional 8 gm. (16 gm. total) were not affected in any way.

At the present time these data may be of academic interest only, because it would not be economically feasible to drill holes and inject systemics in all trees of heavily infested areas. Future developments and modifications of methods of application may overcome the unfavorable economic aspects of the problem.

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Loss of Chlorinated Hydrocarbon Insecticides from Soil Surface in the Field[1]

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ABSTRACT

Four chlorinated hydrocarbon insecticides, namely, DDT, endrin, aldrin, and Shell Development Company SD-4402 (1,3,4,5,6,7,8,8-octahcloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) were applied to the surface of the soil in the field and exposed at least for sometime to relatively high surface temperatures. The rate of loss of activity owing to physical, chemical, and biological processes was measured by bioassay procedure against an eye gnat *Hippelates collusor* (Townsend) and larvae of the mosquito *Culex quinquefasciatus* Say.

After 24 hours of exposure the biological activity of all materials except DDT as measured against eye gnats, had decreased considerably. Approximately 50% of endrin was lost after a 24-hour exposure period as determined by both bioassay methods. The loss of DDT during the same period was about 10%. The overall loss of DDT determined by the gnat bioassay was greater than that obtained by the mosquito larvae bioassay. Greater loss of activity was recorded for Compound SD-4402 by both the mosquito and gnat bioassay procedure than for aldrin. The loss of activity of aldrin and DDT measured by mosquito bioassay method was lower than that measured by the eye-gnat bioassay method for each material. A partial explanation of this phenomenon is offered.

Chlorinated hydrocarbon insecticides have been employed in routine and experimental larviciding programs attempted for the control of *Hippelates* eye gnats in California (Tinkham 1953, Mulla 1958, Mulla *et al.* 1960). These materials are applied to the surface of the soil where eye gnats are breeding in large numbers. Such breeding areas include date gardens, citrus groves, vineyards, and a variety of vegetable and field crop grounds. After application of the larviciding material to the ground, the material is disked under immediately so that loss of the toxicant from the surface of the soil is minimized.

Economic reasons dictate that large areas be treated for eye-gnat control with aircraft or ground equipment.

The treated ground is disked by the owners of the properties treated, and this operation usually requires a day or two for completion. Because of breakdown of farm equipment and other difficulties which may arise, the toxicant applied for eye-gnat control may remain on the surface for 2 or more days. This condition undoubtedly results in loss of some of the material before it is incorporated into the soil for effective gnat control.

This study was undertaken to determine the loss of biological activity (owing to chemical, biological, and physical processes) of four chlorinated hydrocarbon insecticides (DDT, endrin, aldrin, and SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan)) from soil surface in the field. The biological activity of these four materials when used against an eye gnat *Hippelates collusor* (Townsend) in the laboratory (Mulla 1960) was greater than that of other materials of this class of compounds. It is expected that materials disappearing or escaping slowly from the soil surface will have a better chance of being employed in larviciding programs for the control of eye gnats.

METHODS AND MATERIALS.—Coachella fine sand was obtained from virgin desert and air-dried. The dry sand was sifted through a 40-mesh sieve for removal of organic matter and other debris. Five-pound samples of this sand were weighed and placed in wooden-frame boxes covered with canvas on all sides except the top, so that moisture and heat could be transferred from the surrounding soil to the test soil in the box. The soil filled the box to within a quarter of an inch of the top. Ten samples of soil were treated with each insecticide material, and there were two controls for each material. Dilute sprays were prepared from emulsifiable concentrates of aldrin, SD-4402, DDT, and endrin. The sprays were applied to the soil at the rate of 100 gallons per acre, 6 inches deep. This amounted to applying 1.16 ml. of the dilute spray over an area of soil measuring 18 square inches. DDT, endrin, aldrin, and SD-4402 were applied at the rate of 20, 4.8, 18, and 2 pounds of toxicant per acre 6 inches deep, respectively.

After the soil samples had been sprayed with the materials, the boxes were placed in the ground with their tops flush with the surface of the ground in an open vineyard exposed to sunlight throughout the day. This simulated a more severe type of field condition under which insecticides are applied to breeding grounds of eye gnats in the early spring season. Nevertheless, conditions similar to these are encountered at times when breeding grounds are treated for the control of *Hippelates* eye gnats in the Coachella Valley of southern California.

Two samples of each treatment were removed at in-

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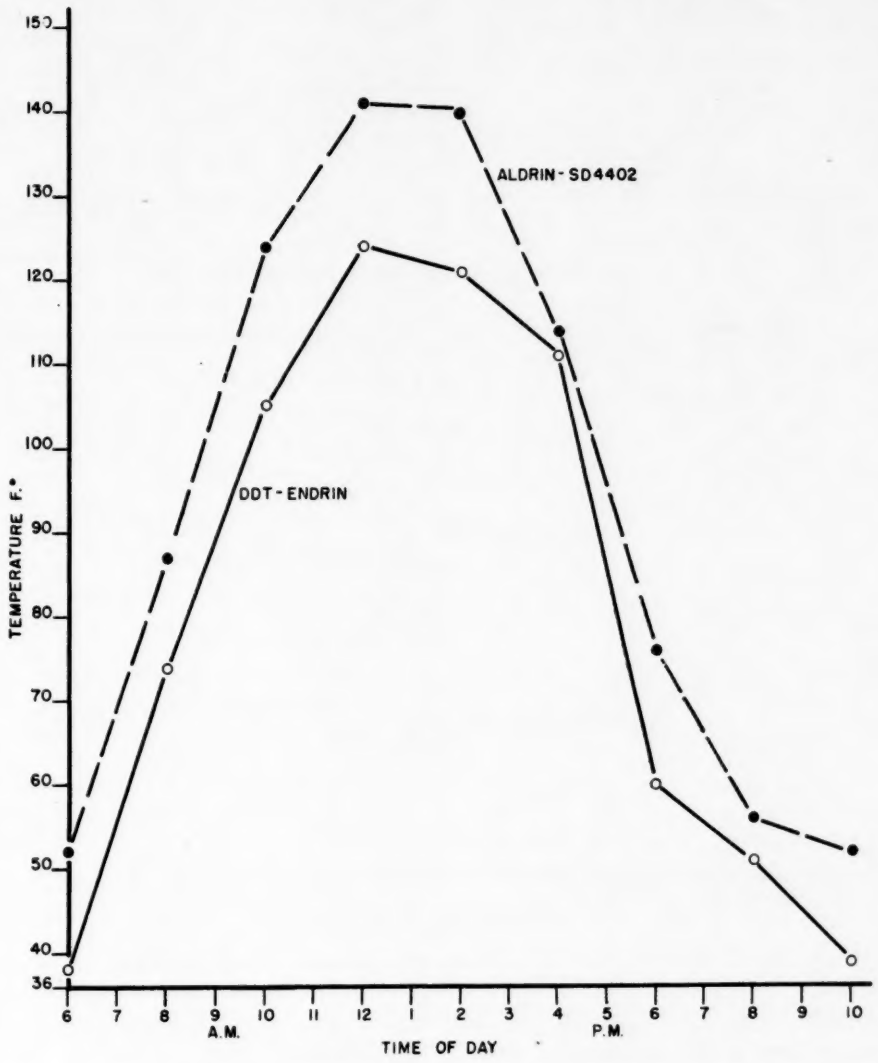

FIG. 1.—Average daily temperatures of soil surface during period of exposure of four chlorinated hydrocarbon insecticides at the surface of the soil.

tervals and transferred to ½-gallon jars which were placed in a refrigerator until the soil was processed for bioassay against the eye gnat *H. collusor* and the southern house mosquito, *Culex quinquefasciatus* Say. Control samples were taken at the beginning and after an exposure period of 24 hours.

Temperature and wind records were kept throughout the period of exposure of the treated soil. Soil surface temperatures were recorded during the exposure period of each set of two materials at approximately 2-hour intervals from 8 A.M. to 4 P.M. daily. Wind and temperature conditions were recorded at 2-hour intervals from sunrise to 10 P.M. 1 day midway in the course of the experiment. The average temperature curves for a 16-hour period are shown in figure 1 for the two sets of tests. Temperatures during the night were below 50° F. and are not included. Winds were infrequent and in no case did wind velocity exceed 5 miles per hour; and wind records are, therefore, omitted here. The weather was clear with full sunshine throughout the duration of the experiments.

Eye Gnat Bioassay.—To each lot of treated and untreated soil an additional ¾ pound of the same type of soil was added to make the total soil quantity 5¾ pounds per treatment. The soil was spoon-mixed thoroughly and organic matter in quantities used in laboratory screening experiments of insecticides against eye gnats (Mulla 1960) was then added to each lot and mixed thoroughly. The soil-organic matter mixture was placed in five 1-pint, narrow-mouthed jars. A total amount of 170 to 180 ml. of water was added to each jar. Two days after addition of the initial 140 ml. of water, 200 eggs of *H. collusor* were added to each jar. The colony of this eye gnat was established in 1957 from gnats collected in the Coachella Valley and was maintained for 24 to 25 generations at the time of this study. After addition of eggs the soil was held at a temperature of 88±2° F. and at a relative humidity of 40% to 50% until the emergence of the

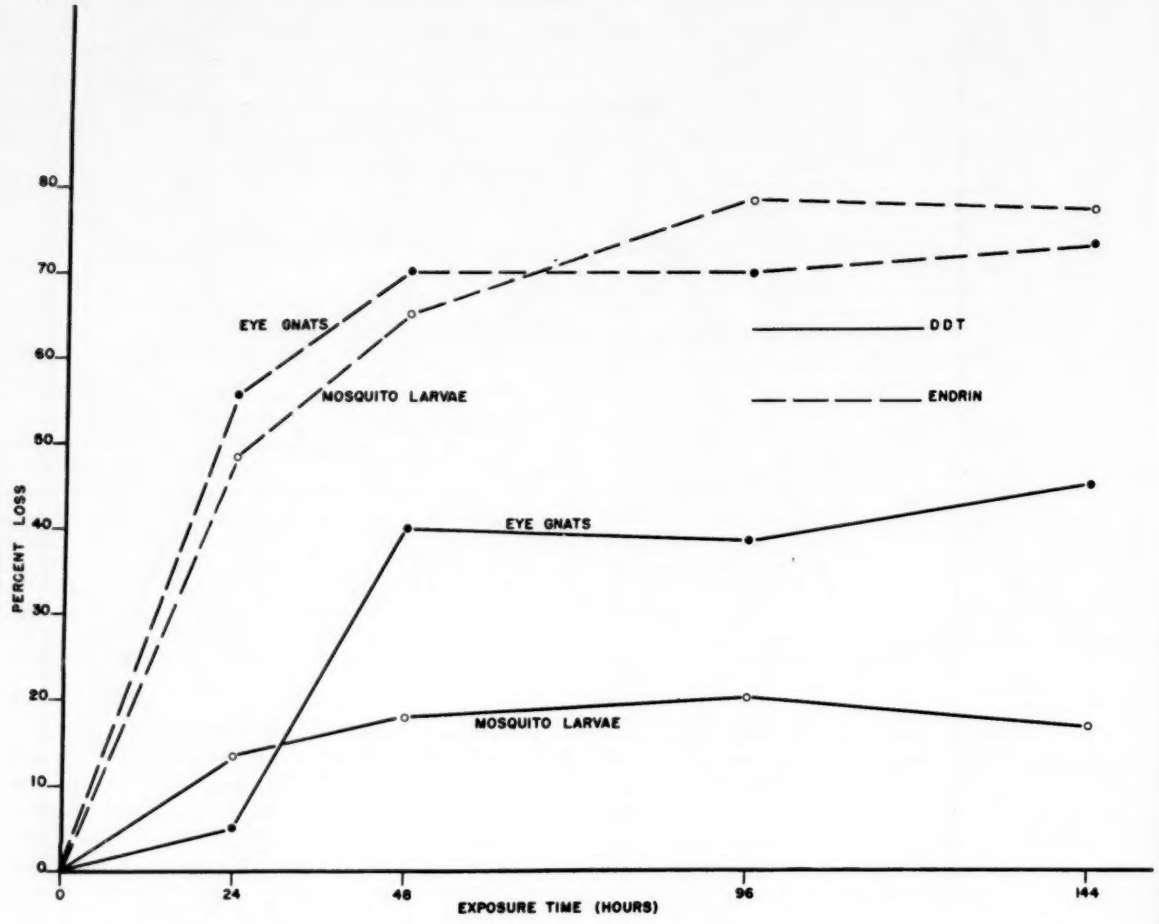

FIG. 2.—Loss of DDT and endrin from surface of the soil as determined by bioassay procedure against eye gnats and mosquito larvae.

gnats was complete. Emerging gnats were counted, and the degree of disappearance of the insecticides was determined from standard dosage mortality lines established for this species (Mulla 1960).

Mosquito Larvae Bioassay.—Each sample of treated and untreated soil was extracted with 1,500 ml. of acetone in stainless steel cans tumbled for a period of 1 hour. After the tumbling, the acetone was filtered through filter paper and the filtrates were stored in a cold room at 40° to 45° F. until needed for bioassay experiments.

Twenty-five 4th-instar larvae of *Culex quinquefasciatus* were transferred to pint-sized cups containing 100 ml. of tap water. The amount of acetone solution to be added per cup was determined by adding a volume of the least exposure (1 hour) treatment to the cup so that a mortality range of 80% to 95% was obtained. All the other insecticidal solutions were then added to the cup at this constant volume, and the mortality of the mosquito larvae was determined after 24 hours. In the longer exposure treatments with SD-4402, the initial volume did not yield mortality of mosquito larvae. This initial volume was then increased and the amount of the insecticide present was calculated. The quantities of insecticides are presented as parts per million (p.p.m.) in the 100 ml. of water in the cup. Standard dosage-mortality lines were utilized in determining the quantities of toxicants present.

RESULTS AND DISCUSSION.—*Tests with DDT and*

Table 1.—Loss of activity of DDT and endrin from surface of the soil in the field as tested against *Hippelates collusor*.[a]

TREATMENT	HOURS AFTER TREATMENT	AVERAGE NO. GNATS/JAR[b]	REDUCTION (%)	TOXICANT RECOVERED/ACRE (LB.)	LOSS (%)
DDT[c]	1	19	82	20.0	0
	24	20	81	19.0	5
	48	41	60	12.4	38
	96	37	65	13.2	34
	144	48	54	11.0	45
Endrin[c]	1	7	93	4.8	0
	24	53	49	2.1	56
	48	84	20	1.4	70
	96	84	20	1.4	70
	144	90	14	1.2	75
Control	1	104	—	—	—

[a] The materials were applied as sprays at the rate of 100 gallons per acre. DDT and endrin were applied at the rate of 20 and 4.8 pounds actual toxicant per acre, respectively.

[b] Five replicates.

[c] Minimum quantities detectable by this method of bioassay are 2.5 and 0.7 pounds per acre for DDT and endrin, respectively.

Endrin.—The rate of loss of activity of DDT from the surface of the soil as measured against the eye gnats (table 1) was lower than that of the endrin. At 24-hour exposure the loss in DDT activity was negligible (5%), while the loss of endrin in the same period of time was 56%. At 48-hour exposure the loss of DDT and of endrin was determined to be 38% and 70%, respectively, of the initial quantity applied. After the 48-hour period further loss was observed for DDT as well as for endrin, but the rate of loss was relatively small.

The results of tests against mosquito larvae showed that DDT lost its biological activity slower than endrin (table 2), a trend noted for these materials earlier. In these tests, the loss of DDT over the entire exposure period was less than that observed in the tests against eye gnats. This situation could be due to biological activity of breakdown products of DDT against mosquito larvae, with little or no activity against the eye gnats. For all practical purposes the loss of endrin seems to follow the same trend in the tests conducted against the two insects.

It is important to note that half of the applied dosage of endrin was lost 24 hours after application (fig. 2) under the conditions of the test. The magnitude of DDT loss within the same period of time was 5% to 13% of the initial quantity applied. To avoid greater losses of both of these materials from the surface of the soil, these materials, particularly endrin, should be incorporated into the soil immediately after treatment. In this respect it is apparent that DDT has certain advantages over endrin, and that more emphasis should be placed on the disking under of the latter material when applied to soil surface against soil-inhabiting insect pests.

Tests with Aldrin and SD-4402.—The loss of activity of both aldrin and SD-4402 is rather high as tested against the gnats. The magnitude of loss of SD-4402 was greater than that of aldrin for each interval of sampling (table 3). Seventy per cent of the activity of SD-4402 was lost after a 7-hour exposure period (9 A.M. to 4 P.M.). The total loss of activity of SD-4402 increased slightly over the rest of the exposure period. The amount of loss of the activity of aldrin at the 24-hour and 7-hour exposure period was substantial as determined by gnat bioassay. After the 24-hour exposure period the activity of aldrin remained at a relatively steady level. This could be accounted for by the fact that aldrin is partly converted to dieldrin (Bollen *et al.* 1958, Gannon & Bigger 1958). It is more likely that the rate of loss of aldrin is a combination of the conversion of aldrin to dieldrin and the subsequent loss of the remaining aldrin and produced dieldrin from the surface. The biological activity of dieldrin against the gnat (Mulla 1960) is lower than that of aldrin.

The relative toxicity of aldrin and dieldrin to eye gnats and mosquito larvae, the test insects in these experiments, is indicated by their (LC50 and LC90) concentrations as shown in table 4.

The toxicity of dieldrin to gnats is almost one-half that of aldrin; for mosquito larvae the reverse is true. With the partial conversion of aldrin to dieldrin, the loss of aldrin as measured against eye gnats is greater, at least in the earlier stages of the exposure period. Conversely, the loss of aldrin under these circumstances, as measured against mosquito larvae, is lower initially (table 5). Appreciable loss of activity of aldrin deter-

Table 2.—Loss of activity of DDT and endrin from surface of the soil in the field as tested against larvae of *Culex quinquefasciatus*.[a]

Treatment	Hours After Treatment	Average Mortality of Larvae[a] (%)	Insecticide Present[a] (P.P.M.)	Average Loss[a] (%)
DDT[b]	1	80	0.0460	0
	24	66	0.0400	13
	48	62	0.0380	18
	96	60	0.0370	20
	144	66	0.0390	16
Endrin[c]	1	100	0.0490	0
	24	81	0.0260	47
	48	50	0.0170	65
	96	16	0.0102	79
	144	19	0.0108	78
Control	24	0	—	—

[a] Average of 3 tests each with 3 replicates per test. The per cent loss was calculated from standard dosage mortality line for each material.
[b] 0.80 ml. of the acetone extract was added to 100 ml. of water per cup. Minimum quantity of DDT detected by this procedure is 0.020 p.p.m.
[c] 0.85 ml. of the acetone extract was added to 100 ml. of water per cup. Minimum quantity detected by this method of bioassay is 0.0070 p.p.m. of endrin.

Table 3.—Loss of activity of aldrin and SD-4402 from soil surface in the field as tested against *Hippelates collusor*.[a]

Treatment	Hours After Treatment	Average No. Gnats/Jar[b]	Reduction (%)	Toxicant Recovered/Acre (Lb.)	Loss[c] (%)
Aldrin	1	7	94	20.0	0
	7	26	78	11.4	30
	24	58	51	6.4	65
	48	40	66	8.4	57
	72	53	55	6.8	62
SD-4402	1	24	80	2.0	0
	7	88	25	0.50	70
	24	67	43	0.66	67
	48	105	10	0.35	82
	72	111	6	0.30	85
Control	1½	117	—	—	—

[a] Dilute sprays were prepared from emulsifiable concentrate containing 2 lb. aldrin and 1.25 lb. SD-4402 per gallon, and applied at the rate of 100 gallons/acre. The materials were applied at the rate of 18 lb. of aldrin and 2 lb. of SD-4402 per acre.
[b] Five replicates.
[c] Per cent loss determined from standard dosage mortality lines for both materials. The minimum quantities of insecticide detectable in pounds per acre are 0.3 lb. and 2 lb. for SD-4402 and aldrin, respectively.

Table 4.—The relative toxicity of aldrin and dieldrin to eye gnats and mosquito larvae.

Material	Mosquito Larvae (P.P.M.)		Eye Gnats (Lb./6″-Acre)	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Aldrin	0.017	0.025	6.2	17.0
Dieldrin	0.009	0.015	12.0	32.0

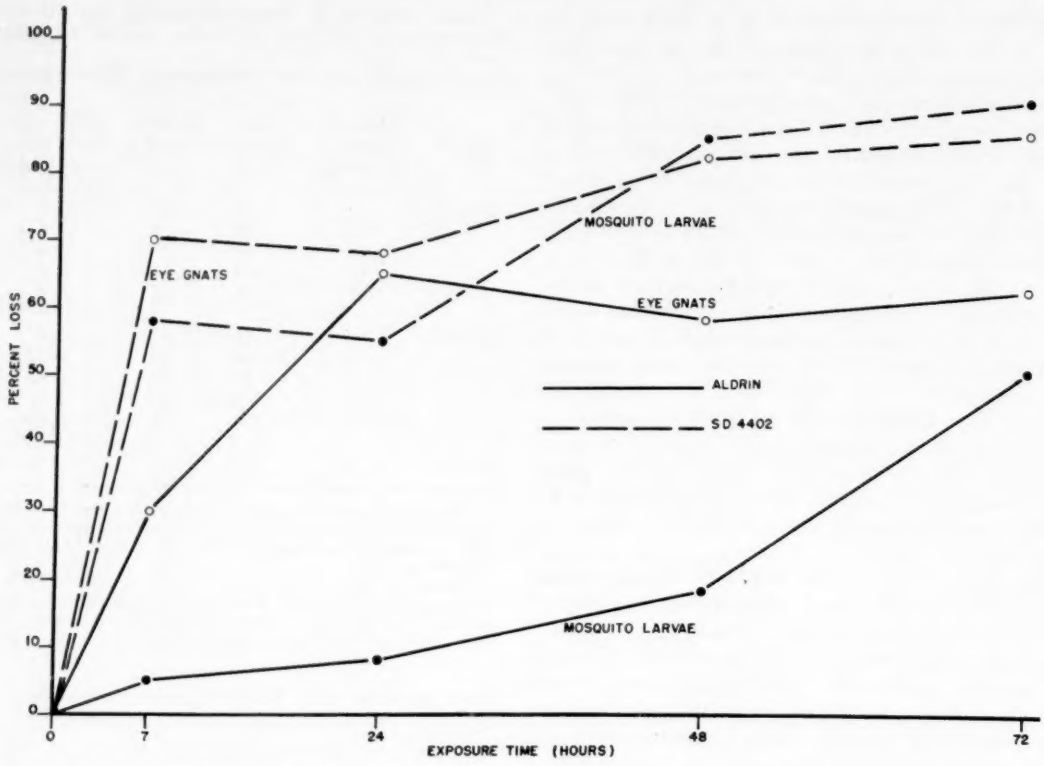

FIG. 3.—Loss of aldrin and SD-4402 from soil surface as determined by bioassay procedure against eye gnats and mosquito larvae.

mined by mosquito larvae bioassay was observed after the 48-hour exposure. Of course, with the elapse of time dieldrin would also begin to disappear from the soil surface but probably at much lower rates than aldrin.

Table 5.—Loss of activity of aldrin and SD-4402 from surface of the soil in the field as tested against lavrae of *Culex quinquefasciatus*.[a]

TREATMENT	HOURS AFTER TREATMENT	AVERAGE MORTALITY OF LARVAE[b] (%)	AVERAGE INSECTICIDE PRESENT[c] (P.P.M.)	AVERAGE LOSS[b] (%)
Aldrin[d]	1	88	0.0215	0
	7	73	0.0205	5
	24	65	0.0190	8
	48	56	0.0178	18
	72	13	0.0107	50
SD-4402[d]	1	98	0.01510	0
	7	57	0.00630	58
	24	67	0.00690	55
	48	22	0.00225	85
	72	1	0.00200	90
Control	72	0	—	—

[a] Aldrin and SD-4402 were applied at the rate of 18 pounds and 2 pounds per acre, respectively.

[b] All averages are based on 3 tests with each treatment replicated 3 times per test.

[c] The amounts of insectides present were calculated from a standard dosage mortality line for each insecticide. The minimum quantities detectable by this bioassay method are 0.01 and 0.002 p.p.m. for aldrin and SD-4402, respectively.

[d] 0.15 ml. of the acetone extract of aldrin-treated soil and 0.5 ml. and 1 ml. of the acetone extract for SD-4402 were added to 100 ml. of tap water in cups to which 25 fourth-instar mosquito larvae were added.

The discrepancy between aldrin contents as measured against gnats and mosquitoes (fig. 3) could be explained in terms of the conversion of aldrin to its epoxide dieldrin. At the 7- and 24-hour intervals the activity of aldrin as measured against eye gnats decreases rapidly owing to the loss of aldrin from the surface and to the conversion of this material to dieldrin, which is less active biologically against the gnats. The rate of loss of activity at the 7-, 24-, and 48-hour intervals, as measured against mosquito larvae, is much lower than that measured by eye-gnat bioassay, because in this case the conversion of aldrin to dieldrin results in higher activity of the residue in the soil.

The rate of loss of activity of SD-4402 follows the same trend by both gnat and mosquito larvae bioassaying procedure (see fig. 3). The loss of activity of this material is thought to be due largely to physical factors such as temperature. In the first 7 hours of exposure, as measured by both testing methods, 58% to 70% of the material was lost. During the next 17 hours of exposure (from sunset to a little after sunrise) no loss was observed. Low temperatures prevailed at the surface of the soil (see fig. 1) and slowed the rate of loss of the test material. Greater loss of activity was again observed in the period from 24 to 48 hours of exposure during which high temperatures prevailed for about 10 hours. On the other hand, the rate of disappearance of aldrin was high during both the diurnal and the nocturnal period, thus suggesting the interplay of chemical and biological factors.

The resolution and accuracy of the bioassay procedures used in these studies are somewhat reduced when slight quantities of the toxicants are to be determined. Such small quantities of residues, except for aldrin, were not expected unless the loss of the applied material approached 75% to 85% of the initial dosage. For aldrin this limit was reached at 50% to 60% loss of the initial dosage. The procedure as a whole has provided a good deal of information on the rate of loss of activity of the four insecticides employed in the tests. The information developed here emphasizes the fact that quick incorporation into the soil of the toxicants used for gnat control, is necessary in order to obtain full benefit of the activity of these insecticides.

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Rearing of Three Spotted Alfalfa Aphid Hymenopterous Parasites for Mass Release[1]

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ABSTRACT

In 1954 the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) first appeared in California and rapidly increased and spread throughout the alfalfa producing areas of the state. In 1955 three species of wasps parasitic on this pest were brought into California from the Mediterranean and Middle East regions where they are indigenous. These parasites were *Praon palitans* Muesebeck and *Trioxys utilis* Muesebeck (Braconidae), and *Aphelinus semiflavus* Howard (Eulophidae).

All three of these species were successfully cultured in the Riverside and Albany insectaries of the Department of Biological Control of the University of California.

The techniques and equipment used in the culture and distribution of the parasites are described, as well as the methods of growing alfalfa in the greenhouse and the culture and maintenance of the host aphid, *T. maculata* in the insectary.

Nearly four million parasites were shipped from the insectaries at Albany and Riverside, chiefly in 1956–57.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), which first appeared in California in January 1954, quickly spread throughout Southern California and in 3 years had infested most of the alfalfa-producing areas of the State (Dickson *et al.* 1955, Smith *et al.* 1956). In 1955 the University of California's Department of Biological Control and the United States Department of Agriculture initiated a search in the Middle East and Mediterranean regions, after it had been determined that this pest was indigenous to the Old World, in the hope of finding effective natural enemies (Van den Bosch 1956, 1957). This effort resulted in the importation of three species of parasites to the quarantine facilities of the University of California at Riverside in 1955. Two were braconids, previously unknown which were subsequently described by Muesebeck (1956) as *Praon palitans* and *Trioxys utilis*. The third is presumed to be the eulophid, *Aphelinus semiflavus* Howard. The three parasites were also shipped to the University of California insectary at Albany. Mass production efforts were undertaken at both places. Subsequently thousands of parasites that were produced in the two insectaries were used in field colonizations and all three species soon became established (Van den Bosch *et al.* 1959). The present paper reports on the rearing techniques devised and utilized at Albany. The same techniques with minor modifications were also employed at Riverside.

In the initial stages of the insectary production program at Albany, quarantine regulations prohibited the rearing of the spotted alfalfa aphid in the insectary. It was therefore necessary to attempt the culture of the introduced species of parasites on aphid species other than *Therioaphis maculata*.

During the period when *T. maculata* was not available as an insectary host it was found that *P. palitans* and *T. utilis* would not attack the pea aphid, *Macrosiphum pisi* (Harris), the potato aphid, *Macrosiphum solanifolii* (Ashm.), the melon aphid, *Aphis gossypii* Glov., or the green peach aphid *Myzus persicae* (Sulz). However *A. semiflavus* did readily attack *M. persicae* and this aphid was used as an insectary host from November 1955 to July 1956. *M. persicae* was cultured on Irish potato plants grown in 6-inch clay pots filled with vermiculite instead of soil. The plants were watered with modified Hoagland's nutrient solution (Ulrich *et al.* 1958) at half

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strength and were cultured under daylight-type fluorescent lamps.

In mid-December 1955, *T. maculata* was found to have spread into Alameda County where the Albany insectary is located and the restriction of the movement of this pest was removed. *T. maculata* was employed thereafter as the chief insectary host of its imported parasites.

In the absence of continuous infestations of *T. maculata* in the Albany area, it became necessary to maintain adequate stocks of the aphid in the insectary the year around. Also since field-grown alfalfa plants became nearly dormant during the winter months it was necessary to grow alfalfa in the greenhouse. A supply of 1200 pots of alfalfa was maintained for the duration of the program.

ALFALFA PLANT CULTURE.—A seed mixture sold by Northrup King Seed Co., was used in the initial alfalfa plantings; later the Calverde variety was substituted. The seed was sown generously in flats filled with unused No. 2 grade vermiculite rather than soil. The flats were watered with the modified Hoagland's solution.

About a month after seeding the secondary leaves were well developed and the seedlings were transplanted into 6-inch clay pots also containing vermiculite. The drainage holes of the pots were covered with sections of 32-mesh lumite screen to prevent the washing away of the vermiculite. Three groups of five seedlings each were planted equal distances apart and about $1\frac{1}{2}$ inches from the side of the pot. Hoagland's nutrient solution was applied generously enough to flow freely from the drainage holes. Plain tap water was used every fourth or fifth watering in order to flush out any excess salts that might have accumulated from previous irrigations with the nutrient solution.

The young plants were cut back periodically to a height of about 3 to 4 inches. The pruning encouraged the rosetting of the plant growth and led to a better stand of stems per pot. As the plants aged they were periodically cut back to a height of 6 to 8 inches. This caused the plants to produce sturdy, upright stems in contrast to the spindly stems of untrimmed alfalfa. Plants cultured during the period of slow growth, *i.e.*, late fall, winter, and early spring, were not ready to be used in the insectary until they were at least 6 months old. During the warmer part of the year at least a 4-month minimum growth period was desirable.

For the large scale watering program the stock nutrient solutions were diluted with water in two open-topped 55-gallon drums which had been covered inside with two coats of nontoxic chemical-resistant paint to prevent rusting.

The nutrient solution was lifted out of the drums by a flexible blade impeller pump and was delivered to the plants through a half-inch garden hose.

Contamination of the alfalfa in the greenhouse by pea aphids was prevented through the use of a TEPP spray applied by a venturi type sprayer attached to a garden hose. Kelthane was used to control spider mites. On one occasion this acaricide was used to control mite infestations on plants supporting stocks of *T. maculata* in the insectary. The mite infestation was eliminated with negligible effect on the aphid stocks. A third insect pest, the leaf miner, *Liriomyza pictella* (Thomson), was controlled by using Dylox.

APHID STOCK CULTURES.—Two pots of alfalfa infested with spotted alfalfa aphid were set up every day. This provided an ample supply of aphids for both stock-room and parasite culture units. Unparasitized aphids taken from *P. palitans* units after the parasite cocooning period had elapsed were sometimes used to supplement the stock room aphid supply in the infesting of plants for the new *Praon* units. Surplus aphids from *Trioxys* cages were used in a similar manner in the rearing program of *T. utilis*.

On about the sixth day after the inoculation of the stock plant, the aphid infestation became so heavy that the aphids began to leave the alfalfa. These wanderers were picked up on "bouquets" of field grown alfalfa sprigs laid at the bases of the pots, and along the edges of the table on which the pots were placed. The "bouquets" were removed each day and replaced with fresh ones. The infested "bouquets" were either used immediately or stored under refrigeration for future use. A "bouquet" consisted of three sprigs of alfalfa about 6 to 7 inches long. The stems were cut evenly, wrapped in a wad of wet absorbent cotton and inserted as a stopper in a water-filled glass vial 3 inches long and $\frac{3}{4}$ inch in diameter. Bouquets prepared in this manner remained in good condition for 2 or 3 days. Furthermore these bouquets remained in excellent condition even after being stored 3 to 4 days in a refrigerator at 80% to 90% relative humidity and 38° to 45° F. before they were to be used. Insect contaminants occurring in the field were largely eliminated from the bouquets by dipping them in a TEPP solution ($\frac{1}{4}$ teaspoon to a gallon of water). These treated bouquets were aerated for 18 to 20 hours before being either used or stored.

The transfer of aphids to new plants was accomplished by pulling the aphid-laden alfalfa stems from the cotton plugs and laying them across the tops of the plants to be infested. The aphids readily moved from the drying bouquet sprigs to the potted plants. At least six heavily-infested stems were placed on each pot of alfalfa in the stock room when a heavy initial infestation was desired.

The stock plants were held in a windowless room at 75° to 78° F. and 45% to 50% relative humidity. Lighting in the room was provided by four light units, each consisting of three 8-foot, 100-watt, daylight type, fluorescent lamps in a reflector mount. Each unit was suspended $2\frac{1}{2}$ feet above a table supporting the plants. The photoperiod of 15 hours was controlled by a time switch. Uninfested plants would remain green and healthy under these conditions but the severe aphid infestations usually killed the plants within a period of 12 days.

The contents of the used pots were emptied into a basket made of $\frac{1}{4}$ inch hardware cloth. This basket served to screen off the alfalfa roots and stems from the vermiculite which could then be reused.

CAGE UNITS.—Two types of culture units were adopted for parasite production. One type was the small sleeve cage, 18 inches wide, 20 inches high, and 17 inches deep, usually a standard item of insectary equipment. The second cage consisted of a light wooden frame 18 inches wide, 30 inches long, and 16 inches high covered on all

sides and top with either organdy cloth, or 32-mesh Lumite plastic cloth. The bottom was left uncovered. This unit was designed to cover six 6-inch clay pots of alfalfa placed on a board platform resting on a table top. The cage was partially raised when necessary to either facilitate servicing the pots, placing and arranging the bouquets, or to introduce parasites.

The sleeve cage units were placed against a ventilating unit (Finney 1960) as the plants required a rapid change of air to prevent a buildup of humidity in the cage. Without this ventilation the leaves of the enclosed alfalfa plants yellowed and dropped. Furthermore a rather heavy growth of mold developed on the honeydew excreted by the aphids which apparently had an unfavorable effect on the parasites.

PROPAGATION OF *Aphelinus semiflavus*.—Under the insectary conditions this parasite proved the easiest of the three to produce. Very large numbers were released in the field in 1955, 1956, and early 1957. *A. semiflavus* could be mass cultured in either of the cages described above. It was necessary to cover the cages with organdy because some of the smaller parasites were able to escape through the 32-mesh Lumite screen.

Three pots of alfalfa were used in each of the small sleeve cages. Each pot was given an initial infestation of 600 to 700 aphid stem mothers. If the aphid stock population consisted largely of immature stages, greater numbers were used. About 30 females of *A. semiflavus* were initially placed on each pot. Four days later an additional 20 females per pot were added. The pots were set in glazed 6-inch saucers resting on blocks so that the tips of the alfalfa plants just touched the glass top of the cage.

Under insectary temperatures of 75° to 78° F. and relative humidity of 45% to 50% the black mummified aphids began to appear on the sixth day. Parasite emergence took place on the 12th day.

The evident faculty of *A. semiflavus* females to discriminate between parasitized and unparasitized hosts made for excellent biological economy and a high percentage of parasitism especially if the parasite-host population was optimal. Dissections revealed that when such was the case only rare instances of superparasitism occurred even when parasitism approached 100%.

If the aphid population was initially heavy more aphids were likely to escape parasitism. The isolated host or one on the periphery of crowded groups was much more vulnerable than those within the crowded area. This was because the female *A. semiflavus* seems to prefer the stable footing of plant surface in parasitizing the aphid host.

If, on the other hand, the population of parasites is relatively high the successive visitation of the parasites to the aphids disturbs them. This causes them to move about, on or off the plant, so much of the time that they become weak and may perish. This factor increases in intensity as the host becomes less abundant until in some instances very few aphids survive.

The percentage of males was usually quite low, often being only 3% to 5% of the total population. This was most likely caused by favorable rearing temperatures since Schlinger & Hall (1959) noted that male production increased to as high as 99% at temperatures of 78° to 85° F. For purposes of determining the production per female used as "sting" insects the male population was figured as 10% of the total number introduced into the cages.

The production of *A. semiflavus* per 6-pot organdy cage was at times as high as 30,000, however, the average yield was about 18,000 to 20,000 per cage. To obtain high yields the use of "pick up" bouquets was very important in salvaging the aphids, many of them parasitized, that had dropped to the floor owing, in part, to the action of the parasites and the movement of dislocated aphids on the plants. Most of these fallen aphids would find, and settle on, the bouquets laid at the base of the pots. When the bouquets became heavy laden with aphids the sprigs were removed from the vials and laid back on the potted alfalfa.

Collecting adult parasites for field release was quite laborious. This operation was avoided, however, by cutting and shipping the alfalfa plants bearing unemerged cocooned parasites. Parasites thus transported were more vigorous and had greater longevity than those collected, stored for a day or more and then shipped to the field as adults.

The alfalfa was cut on the 10th day and spread out to dry for 24 hours. Most of the remaining living aphids were unparasitized and were shaken from the hay before it was shipped. The alfalfa was loosely packed in 14″ ×14″×18″ corrugated cardboard cartons with a few handfuls of dry excelsior included to lessen the chance of compression and to absorb some of the excess moisture. As most of the shipping destinations were only 24 hours distant from the insectary the material arrived in good condition.

THE PROPAGATION OF *Praon palitans*.—This braconid was for the most part cultured in 6-pot cage units covered with the Lumite screen. A moderately heavy infestation (either 600–700 stem mothers or 1000 immature aphids) was placed on each pot of alfalfa. From 20 to 30 female *P. palitans* per pot were released in the cage as "sting stock." The male and female parasites used for this purpose were collected in lots of 20 to 30 in glass tubes streaked lightly with honey. They were given opportunity to feed and mate for several hours before being placed in the cage. On about the second or third day when the aphids began to move off the plants, 10 or more pick-up bouquets were laid around the inside of the cage and between the pots.

At 75° F. and 45% to 50% humidity, *P. palitans* larvae commenced cocooning on the sixth day and adults began to emerge on the 12th day. On the 11th day the alfalfa was cut and placed in a large sleeve cage where the emerging adult parasites were collected by aspiration. Parasite emergence usually continued for 7 to 8 days with peak emergence occurring on the third day. Honey was streaked on the glass top of the cage as a food supply for the adults. This species was easily collected with a mouth aspirator which had less damaging effects on the parasites than the power aspirator as described for *A. semiflavus*. Parasite adults were collected in open-end Pyrex glass tubes 6 inches long and an outside diameter of 1 inch. A No. 4 two-hole rubber cork was used with

the aspirator head and a tight fitting wad of cotton plugged the opposite end of the tube. When a tube had been filled with its quota of parasites the aspirator cork was removed and replaced by a cotton stopper.

After allowing 2 hours for mating and feeding in the tubes, the parasite adults were anesthetized with CO_2 and emptied into flat, cylindrical, half-pint cardboard ice cream cartons for shipment to the field. The cartons were streaked on the sides and top with honey. A loose ball of excelsior was included in the carton to create additional resting surface for the parasites. Three or four hundred adults were shipped in each carton.

From March 1957, to the termination of the project in October 1957, *P. palitans* was shipped to the field on alfalfa cut on the 10th day after the initial sting or just prior to parasite emergence. On the 11th day the remaining aphids, which were nearly all unparasitized, were shaken from the semidry host plant material. The parasites then were shipped in the same manner as described for *A. semiflavus*.

The best yield of parasites was obtained from well-matured plants more than a year old, some of which were in use for the second time. This type of plant supported, without premature loss, a heavier infestation of aphids which in turn permitted the use of 180 to 200 females per unit as "sting" insects. Forty-five units in which the older, hardier plants were utilized produced an average of 6000 parasites per 6-pot unit as compared with 3000 parasites per cage unit when a more tender type of alfalfa was used.

PROPAGATION OF *Trioxys utilis*.—This parasite was the most difficult of the three species to propagate in the insectary. The ovipositional activity was intense; a single female often attacked 30 or more aphids in 10 to 15 minutes causing most of the attacked hosts, especially the adults, to jump from the plant or quickly crawl away. In spite of this aggressive ovipositional activity the increase per female was relatively low averaging not more than 6- to 7-fold with a sex ratio of 1:1. This rate of increase prevailed whether one female or 25 were used as "sting" insects in a sleeve cage. When more than 30 were used the rate of increase per female tended to be even lower.

Under humid conditions (60% relative humidity and higher) *T. utilis* adults spent most of their time in stroking their antennae, wings and legs rather than ovipositing. However, by the use of the ventilating unit mentioned above the humidity in the cage was reduced to room level even when as many as three pots of alfalfa were used in a cage.

As mentioned above the ovipositional activity of the *T. utilis* females caused many of the larger aphids to leave the plant. This made the use of pickup "bouquets" a prime necessity as some of the wandering aphids were parasitized and would otherwise have perished prematurely on the cage floor.

In routine production at Albany the pots of lightly infested alfalfa were placed in a small sleeve cage set against the ventilation unit. Twenty-five *T. utilis* females that had been allowed to feed and mate for several hours were introduced into each cage. Honey was streaked on the glass of the cage to provide food for the adult parasites. At least one pickup "bouquet" was laid on the cage floor. When the "bouquet" sprigs were well laden with aphids they were pulled from the cotton in the vial and laid across the potted plant. The plants were well watered when placed in the cage, and thereafter watered as needed to maintain a damp subsurface condition.

The mummified aphids began to appear on the sixth day and the *T. utilis* adults commenced to emerge on the 10th day. On the ninth day the alfalfa was cut and laid on the cage floor or hung on hooks on the cage walls. The pot was then removed, as well as the saucer, unless there were many mummified aphids attached. Most of the live aphids, largely unparasitized, were removed from the cage just prior to parasite emergence, otherwise the *T. utilis* would become less effective in the field by wastefully ovipositing in the residue of aphids remaining in the cage.

Comparable production of the parasites, *P. palitans* and *A. semiflavus*, were obtained at both the Albany and Riverside insectaries. *T. utilis* on the other hand was propagated in larger numbers at Riverside. This was primarily caused by the larger number of cage units used on the production line. Two other factors appear to have contributed to the increased productivity of this parasite at Riverside: (1) the temperature at which the parasite was reared at Riverside ranged from 70° to 75° F. while at Albany it ranged from 75° to 78° F. and at times on sunny days rose to above 80° F.; (2) the use of tougher stemmed, mature alfalfa plants at Riverside which had been dug from nearby fields, transplanted into large clay pots, and fumigated with methyl bromide after having been stripped of most of their leaves, whereas at Albany the alfalfa was all greenhouse grown, tender and succulent for the most part.

According to cooperative reports by D. W. Jones, excellent production of *T. utilis* was obtained at the U. S. Department of Agriculture Laboratory at Moorestown, New Jersey, where the yellow clover aphid, (*Therioaphis trifolii* (Monell)) was used as a host. Good parasitism could also be obtained when the sweet clover aphid, (*Therioaphis riehmi* (Börner)) was used as a host (Schlinger & Hall 1959).

During 1956 and 1957 the shipping of parasites began in March and ended in October of each year. Insectary production continued however on a maintenance level the remainder of the year. Many of the parasites reared during this interim period were used in various types of laboratory experiments; others were used as sting stock and the remainder were discarded as surplus.

More than 45,000 parasites were shipped from the insectaries at Albany and Riverside for release in California late in 1955: 13,000 *A. semiflavus*, 26,000 *P. palitans*, and 6,000 *T. utilis*. In 1956 and 1957 2,265,000 *A. semiflavus*, 1,414,000 *P. palitans*, and 213,000 *T. utilis*, or a total of 3,892,000, were shipped to the field. Roughly 75% of these were released in California; the rest were consigned to other states.

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Biology of the Black Cherry Aphid in the Willamette Valley, Oregon[1]

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ABSTRACT

A study of the biology of the black cherry aphid (*Myzus cerasi* (F.)) was made during the 1956 season. Although it was possible to control this aphid early in the spring, heavy reinfestations often developed during the late spring and early summer. During this study there was evidence of considerable movement of the winged summer migrants from one cherry tree to another to feed and reproduce. It was believed previously that these forms migrated directly to alternate host plants during the summer. These aphids were not found on alternate host plants in 1956, although observations in the present study indicated that no males were produced on cherry.

It was estimated, on the basis of a field-rearing experiment covering part of the season, that a total of 12 generations, plus a partial 13th, were produced during the entire season. In the same experiment the developmental period, from birth to reproduction, ranged from 11.3 days in the 9th generation to 35.9 days in the 12th generation. The average number of nymphs per female per day ranged from 1.1 to 1.9.

The following new predators and parasites were recorded: *Hippodamia 5-signata ambigua* Muls., *Cycloneda polita* Csy., *Syrphus vitripennis* Meigen, *Ephedrus nitidus* Gahan, *Aphidius* (*Lysiphlebus*) *knowltoni* Smith.

A study of the biology of the black cherry aphid (*Myzus cerasi* (F.)) was conducted in 1956. Experiment station entomologists had noted that infestations developed consistently during the late spring and early summer in many cherry orchards throughout the Valley, even though standard spray recommendations were followed during the dormant and green tip stages of tree development. Since the populations in the treated orchards were very low until late spring, dispersal of summer migrants from nearby untreated plantings appeared a likely source of the infestations in the sprayed orchards. This supposition was contrary to the general opinion that the winged forms of this aphid, which appeared in the spring or early summer, migrated directly to alternate host plants without first dispersing to other cherry trees. Observations were made at Corvallis, Oregon, to obtain information on this question.

SEASONAL HISTORY.—In contrast to the apple aphid (*Aphis pomi* DeG.), eggs of the black cherry aphid are not found in great abundance in the field. Counts made during the 1956 season indicated an average of approximately 20 eggs per 100 shoots, each shoot being approximately 2 to 3 feet long. The stem mothers, which hatched from the overwintering eggs, first appeared between March 15 to 20, while the buds were swelling. By March 28, when the buds were in the green-tip stage, all eggs presumably had hatched, since none was found after an extensive search of several orchards. The stem mothers began reproducing on April 18, at the time the cherry trees were in full bloom. One week later the first leaf curl injury was found.

The progeny of the stem mothers (apterous viviparae) began reproducing May 3, approximately 15 days after birth. This apterous form persisted on cherry trees until fall. Alate forms (summer migrants) of the fourth generation were observed in small numbers on May 29 and appeared to reach peak abundance by the latter part of June.

Natural enemies of the aphid were present in large numbers by late May. Aphids were reproducing at such a rapid rate, however, that they were not being held in check. The aphid population remained high through the harvest season until July 19, at which time the population was suddenly reduced, following 3 or 4 days of unseasonably hot weather. Most of the aphids that escaped the apparent effects of the hot weather were located in large trees where they were shaded sufficiently for protection from the direct rays of the sun. Thereafter, until late in the season, aphids were found only in small isolated colonies on water sprouts and on other parts of the tree that remained in a succulent condition.

As fall approached, the apterous forms that had per-

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sisted on the cherry trees through the summer began giving birth to alate forms (winged sexuparae). In 1956, these first appeared in small numbers in the 10th generation. Approximately one-half of the 11th and 12th generations were alate forms, similar to the summer migrants. These first appeared on September 15 amid colonies of apterous forms. Within a few days they began to disperse to other cherry trees, where they infested leaves of all ages throughout the tree. Dispersion of the winged forms continued through October until they were well established in nearly all cherry orchards in the area, on both sweet and sour cherries.

Progeny of the winged sexuparae, which were to become the egg-laying females, were observed in the first nymphal stage on September 22. Subsequent cool weather slowed the rate of development of the nymphs and the first adult sexual females were found on October 20. The adults continued feeding on the cherry foliage for approximately 2 weeks before they began mating. The first males were observed mating with the wingless oviparous females in several orchards on November 2. To determine the sex ratio, field counts of 100 leaves were made at this time, and again on November 5. These counts indicated the sex ratio to be approximately 2:1 in favor of the females. The first overwintering eggs were found November 5. Rearing experiments indicated that an average of approximately 4 to 5 eggs per female were deposited. The oviposition period lasted only a short period. A heavy leaf drop occurred in most of the orchards between November 5 to 10, resulting in greatly diminished numbers of these forms. The remnants of the aphid populations were killed by frost on November 19.

ALTERNATE HOSTS.—Ross (1918a,b) and other workers have reported numerous alternate host plants for the black cherry aphid, including wild peppergrass, shepherds-purse, water cress and other crucifers. Between the middle of July and late October, numerous attempts were made to locate this aphid on an alternate host in the field. These efforts were unsuccessful even though shepherds-purse and other crucifers were found in abundance in and around many infested cherry orchards.

Late in August, several shepherds-purse plants were transplanted to flower pots and cages placed over the pots. Weak colonies of black cherry aphids were established and maintained in the cages until late September, when they all died.

Approximately 200 winged individual aphids were produced in rearing experiments on cherries during October and November. Not a single male was found among these aphids. These findings lead the writer to support Ross (1918a,b) who states that no males are produced on cherry, these forms being produced exclusively on alternate host plants in the fall and that they later fly to cherry to mate with the wingless females. Alternate host plants, which have not been found in the Willamette Valley, apparently are necessary for the male aphid to complete its life cycle.

DISPERSION AND MIGRATION HABITS.—While it is generally believed that the summer migrants fly to alternate hosts, evidence was obtained that considerable movement of these forms occurs from one cherry tree to another, where feeding and reproduction occur. Much of the aphid rearing work was carried out in a small orchard containing four rows of young cherry trees. Rows were spaced 15 feet apart, each containing approximately 20 trees. Until late in the summer, a single outer row was used for rearing experiments. All trees in the remaining rows were examined frequently for the presence of aphids during the spring and early summer. None was found until July 7, when small numbers of summer migrants began appearing on these trees. Many of the migrants inhabited a single terminal for only a short period, giving birth to 1 to 8 young, and then flying to another terminal, or to another cherry tree, before producing additional offspring.

Table 1.—Black cherry aphid infestations on treated and untreated sweet cherry trees before and after appearance of winged summer migrants.

CONDITION	TOTAL TREES	INFESTED TIPS PER TREE	INFESTED TREES (%)
	May 24, 1956		
Treated	50	1.5	12
Untreated	13	12.8	77
	June 22, 1956		
Treated	50	14.1	68
Untreated	13	99.2	100

Although a heavy infestation of aphids appeared to be developing at this time, it failed to materialize. Coincident with the unseasonably hot weather of July 19, the aphid population in this orchard was destroyed.

Further observations on aphid movement from tree to tree were made in a commercial cherry orchard in which insecticides were being evaluated for aphid control.[4] Trees in this orchard were sprayed during the dormant stage to prevent egg hatch. A scattering of untreated check trees was left in the block. On May 24, before the winged migrants began to appear, most of the treated trees were entirely free of aphids, while nearly all the untreated trees were heavily infested. By June 22, nearly all previously uninfested trees were heavily infested, and numerous winged forms were present. Results of counts made on these dates are shown in table 1. These observations indicate that considerable movement of the winged summer migrants from one cherry tree to another takes place, even though there may be a relationship between the insect and alternate host plants.

REARING EXPERIMENTS.—Rearing experiments were conducted in the field and to a limited extent in the laboratory. Aphids were reared in the laboratory in Munger cells (Munger 1942) which had been modified and used previously by other entomologists at Oregon State College. In the field, the insects were reared in "clip-on" leaf cages (Raine 1956) on young cherry trees 1 to 3 years old. In both techniques, newborn nymphs were placed singly in the cages and observed once daily to determine the rate of development and reproduction.

The field-rearing experiments were started on June 14. On the basis of prior data based on field observations and from subsequent rearing experiments, it was estimated that field rearing was begun with individuals of the sixth generation. Data were compiled for each subsequent generation, except the seventh, which was destroyed prematurely by hot weather. Seven generations, plus a partial eighth, were obtained from these rearing experiments.

[4] The author assisted S. C. Jones, Experiment Station Entomologist, in these studies.

Table 2.—Longevity and reproduction rate of the black cherry aphid under laboratory and field conditions.

GENERATION	NO. OF APHIDS	AVERAGE NO. DAYS BIRTH TO REPRODUCTION	REPRODUCTION PERIOD (DAYS)	AVERAGE NO. NYMPHS PER DAY
		Laboratory		
—	6	10.0±1.7	3.3±1.5	1.4±0.8
—	7	8.7±0.5	9.8±3.9	2.2±0.4
—	8	8.1±1.2	7.6±2.6	1.8±0.4
		Field		
6	18	15.7±2.6	13.8±4.0	1.9±0.8
7[a]	—	—	—	—
8	5	25.7±7.1	4.3±4.0	1.1±0.3
9	7	11.3±1.6	12.1±8.8	1.4±0.6
10	6	17.0±2.6	10.3±5.7	1.4±0.3
11	10	15.7±2.4	17.6±4.6	1.1±0.3
12	13	35.9±4.1	7.1±4.5	1.1±0.3

[a] Aphids destroyed by hot weather. No data obtained.

Since the experiments probably began with the 6th generation, it was estimated that a total of 12 generations, plus a partial 13th were produced during the entire year. Data compiled for both laboratory and field rearing experiments are shown in table 2. Standard deviations are given for each mean.

NATURAL ENEMIES.—Coccinellids appeared to be the most important group of predators of the black cherry aphid in the Willamette Valley, from the standpoint of abundance and because aphids were attacked by both larvae and adults. The larvae of these beetles were particularly voracious in their feeding habits. Half-grown larvae were observed to consume as many as 30 to 35 aphids in a 24-hour period.

Syrphid larvae appeared to be next in importance as predators. Larvae of this group were found on cherry trees from early June until late October.

Chrysopid larvae were a third important group of predators of the aphid. These did not appear until late summer during the 1956 season, however, and were not found in great abundance.

During late May, it was noted that many of the aphids in the larger colonies were parasitized. In the early stages of parasite development the aphids appeared swollen and distended. Several leaves with colonies containing such individuals were brought to the laboratory and placed in cages. Within a few days, numerous braconid parasites were recovered. On June 9, thirty leaves were examined for parasitized aphids in an attempt to determine the relative importance of these parasites. Approximately 3,600 aphids on these leaves were examined and about 17% were parasitized.

Specimens of predators and parasites were identified as follows:[5] Coccinellidae: *Adalia bipunctata* (L.), *Hippodamia 5-signata ambigua* Muls., *Cycloneda polita* Csy.; Syrphidae: *Syrphus vitripennis* Meigen; Chrysopidae: *Chrysopa* sp., *plorabunda* group; Braconidae: *Ephedrus nitidus* Gahan, *Aphidius* (*Lysiphlebus*) *knowltoni* Smith.

With the exception of *Adalia bipunctata* (L.) and *Chrysopa* sp., this list represents a new record of predators and parasites of the black cherry aphid, as far as known.

Several secondary parasites belonging to the family Pteromalidae were identified as *Pachyneuron* sp.

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[5] Identifications made by the Insect Identification and Parasite Introduction Branch, U. S. Department of Agriculture and the Division of Insects, Smithsonian Institution; Coccinellidae by E. A. Chapin, Syrphidae by P. H. Arnaud, Chrysopidae by S. Parfin, Braconidae by C. F. W. Muesebeck, and Pteromalidae by B. D. Burks.

An Evaluation of the Rate of Reproduction of the Two-Spotted Spider Mite Reared on Gibberellin-Treated Bean Plants[1]

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ABSTRACT

An investigation was established to determine if populations of two-spotted spider mites, *Tetranychus telarius* (L.), were affected when reared on Tendergreen variety of snap beans treated with gibberellin. Five treatments of gibberellin were evaluated utilizing the excised leaf-disc method. Four mite-disc arrangements were included in each treatment. There were significant reductions in mite populations on plants treated with certain gibberellin applications. There appeared to be differences resulting from factors other than direct plant growth. The rate of reproduction of mites was low on treated plants, but rose rapidly and became almost normal when these mites were returned to untreated plants. Mites raised on untreated plants and transferred to treated plants showed a drop in reproduction within one generation.

The correlation between mite populations and the mineral composition of soil and plant tissue has been studied by numerous investigators. Rodriguez & Neiswander (1949) observed a positive correlation between the concentration of soluble salts in greenhouse soils and mite populations. Garman & Kennedy (1944) reported a similar correlation between soil fertility and mite reproduction on beans and peaches. In a detailed evaluation of the effect of several major elements on mite populations Rodriguez (1951, 1958) found that the balance of various elements in the host plant was the important

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factor determining the rate of mite reproduction. The morphological characters and the nutritional balance of many plants are altered when these plants are treated with gibberellin (Morgan & Mees 1956, Stowe & Yamaki 1957, and Wittwer & Bukovac 1957). The purpose of this experiment was to determine if mite populations were affected when bean plants were treated with gibberellin.

PROCEDURE.—*Experiment A, Part 1.*—Experiment A was conducted during January and February 1958 in a constant-temperature room with artificial light. The temperature was maintained at 72° F. ± 2°. The illumination consisted of 450-foot candles of incandescent and fluorescent light at the plant level. Eighteen standard 5-inch clay pots were filled with potting soil, sterilized in an autoclave, and planted with six Tendergreen bean seeds per pot.

The beans emerged six days after planting. At this time nine of the pots were selected at random and the beans treated with a three-second application of gibberellin aerosol. This was the equivalent of a 15 p.p.m. aqueous solution sprayed to run off. The other nine pots of beans were maintained as a control. Five days later a second application was applied to the treated beans. On the succeeding day, 10 pots of beans were selected (five from the control pots and five from the treated pots) and infested with mites. A strand of Tanglefoot was applied to the stem of each plant to prevent migration from the host.

Four days after infestation, the third and final application of gibberellin was made to the nine pots of beans previously treated. This included the five pots that were infested with mites. At this time, the treated plants were more than twice as tall and were much lighter in color than the untreated ones. On the day following the third gibberellin treatment, 10 leaf discs seven-eighths inch in diameter were cut from the treated plants. A similar sample was obtained from plants growing in the four control pots that were not infested with mites.

A slight modification of the detached leaf-disc technique described by Rodriguez in 1953 was followed. Each cut disc was fastened to a small pencil eraser with a pin and floated in a petri dish containing a 2% sucrose solution. Each disc was then infested with five female mites which had been taken from the plants infested 5 days earlier. These were placed on the discs according to the following arrangements: (1) Mites from control plants put on leaf discs from control plants, (CM-CD); (2) mites from control plants on leaf discs from treated plants, (CM-TD); (3) mites from treated plants on leaf discs from control plants, (TM-CD); and (4) mites from treated plants on leaf discs from treated plants, (TM-TD). The petri dishes containing the discs were then placed under lights and left for 10 days. Water was added to the dishes daily to replace that lost by evaporation.

Experiment A, Part 2.—All except two of the bean plants were removed from each of the eight remaining pots in Part 1 (four uninfested controls and four uninfested treated plants). A single leaf on each plant was sealed off with Tanglefoot. Five female mites from the infested plants were then placed on each leaf, according to the same arrangement as described for Part 1.

Experiment B.—This experiment was arranged in the same general manner as Experiment A but with several modifications. There were 15 different arrangements plus a control in contrast to the three arrangements and a control in Experiment A. Gibberellin was applied to the plants as: (1) a 3-second aerosol spray applied once, (2) a 3-second aerosol spray applied twice at an interval of 5 days, (3) a 3-second aerosol spray applied three times at intervals of 5 days and (4) an 1,100-p.p.m. slurry treatment applied on the seeds.

The two applications of a three-second spray were duplicated on two sets of bean plants. From one set, discs were taken 9 days after the last application. From the second set of beans, discs were removed immediately after the second spray. At this time, the effects of gibberellin on the plants were just becoming apparent. Experiment B was conducted during April and May and all plants were grown in the Plant Science Greenhouse under normal sunlight. Aside from these differences, the experiment was comparable to that of Part 1 of Experiment A.

The data from Experiments A and B were treated separately but in the same manner. The mites and eggs on the replicated discs or leaves of each mite-disc arrangement were counted and the mean of the total progeny for each arrangement was calculated. An analysis of variance was then made to determine if there was a significant difference between the treatments or arrangements.

RESULTS.—In Part 1 of Experiment A mites raised on control plants and placed on treated discs (CM-TD) and mites raised on treated plants and placed on treated discs (TM-TD) had a significantly lower rate of reproduction than mites raised on control plants and placed on discs removed from control plants (CM-CD) or mites raised on treated plants and placed on discs from control plants (TM-CD) (table 1). No other significant differences were evident in Part 1 of Experiment A.

Results of Part 1 of Experiment A indicated that the reproductive capacity of mites was inhibited when mites were reared on discs removed from plants that had been treated with gibberellin. This inhibition in reproduction was not permanent. When mites were transferred from treated plants to control discs, the reproduction rate returned nearly to normal in the next generation. The reverse was also true, when mites living on control plants were transferred to discs from treated plants, reproduction dropped in the next generation.

Part 2 of Experiment A (table 1), in which mites were placed on growing leaves rather than on excised discs, showed results similar to Part 1 with the following excep-

Table 1.—Mean number of progeny from mites placed on leaf discs and leaves from bean plants sprayed three times with gibberellin in experiment A.

MITE DISC OR LEAF ARRANGEMENT[a]	PART 1, PROGENY PER DISC	PART 2, PROGENY PER LEAF
CM-TD	17 a[b]	34 c
TM-CD	110 b	148 a
TM-TD	27 a	68 b
CM-CD	121 b	153 a

[a] In describing arrangements, C = control, T = treated, M = mites, and D-disc or leaf.

[b] If two treatments do not have a common letter, the treatments are significantly different at the 5% level. If two treatments have a common letter, they are not significantly different.

Table 2.—Mean number of progeny from mites placed on leaf discs removed from bean plants treated with different applications of gibberellin in experiment B.

TREATMENT	MITE-DISC ARRANGEMENTS[a]	PROGENY PER DISC
Gibberellin slurry	CM-TD	123 ab[b]
	TM-CD	125 ab
	TM-TD	129 ab
One 3-second aerosol spray	CM-TD	129 ab
	TM-CD	111 b
	TM-TD	119 ab
Two 3-second aerosol sprays	CM-TD	110 b
	TM-CD	156 ab
	TM-TD	113 b
Three 3-second aerosol sprays	CM-TD	22 cd
	TM-CD	130 ab
	TM-TD	9 d
Two 3-second aerosol sprays cut at half growth	CM-TD	32 cd
	TM-CD	159 a
	TM-TD	55 c
Control	CM-CD	175 a

[a] In describing arrangements, C = control, T = treated, M = mites, and D- = disc.

[b] If two treatments do not have a common letter, the treatments are significantly different at the 5% level. If two treatments share a common letter, they are not significantly different.

tion. The reproduction of mites raised on treated plants and placed on treated leaves tended to be higher than that of mites raised on control plants and transferred to treated leaves.

In Experiment B an analysis of variance was made on the data from all 16 arrangements (table 2). This was done not only to compare the reproduction within each type of gibberellin application, but also to evaluate the effect on populations resulting from the different gibberellin treatments. Because of the difficulty involved in discussing all 16 arrangements concurrently, the results of the mite arrangements for only one type of gibberellin treatment will be considered at one time in the first part of this discussion. A comparison of the effects of the various treatments on mite populations will be discussed later.

There was no significant difference between the number of mites found on the discs of the three arrangement variations within the slurry treatment. Nor was there a difference between any of the mite arrangements and the control. These data indicated that there was a trend toward a lower mite population on plants where the seed had received the slurry treatment of gibberellin.

The analysis of the mite data from the plants receiving a single aerosol spray showed that there were no significant differences between any of the mite disc arrangements within this treatment; however, there were significantly fewer mites produced where treated mites were placed on control discs (CM-CD).

The data from the two three-second aerosol spray treatments applied 5 days apart indicated: (1) Mites raised on control plants and placed on discs from treated plants (CM-TD) had significantly lower numbers of offspring than mites raised on control plants and placed on discs from control plants, (CM-CD); (2) the reproduction of mites raised on treated plants and placed on treated discs (TM-TD) was significantly lower than that of mites raised on control plants and placed on control discs (CM-CD).

The analysis of the mite data resulting from the 3-second aerosol spray applied three times at 5-day intervals indicated that all mites transferred to treated discs showed a significant drop in reproduction when compared with mites raised on control plants and placed on control discs. Mites raised on treated plants and placed on discs from control plants (TM-CD) had a significantly higher rate of reproduction than either mites from treated plants placed on treated discs (TM-TD) or mites from control plants placed on treated discs (CM-TD). This part of Experiment B was a repetition of the first part of Experiment A and shows similar results.

The last group of plants to receive a treatment of gibberellin was sprayed twice with a 3-second aerosol spray. The discs from these plants differed from those of the other plants receiving the same treatment in that they were cut out immediately after the second spray. This was before the plants had achieved full growth. Analysis of the data for the mites and eggs of this second two-spray treatment showed results comparable with those from plants receiving three gibberellin sprays.

Throughout Experiment B, the analysis of variance for the mite data showed varying levels of mite response to different types or rates of treatment with gibberellin. For the slurry treatment and for the one-spray treatment there was no significant drop in the mite population on the discs with the exception of the mites from the aerosol-treated plants that were transferred to the untreated control.

Discs that had received two sprays of gibberellin showed a significant reduction in the numbers of mites and eggs. Discs from plants that had received two sprays of gibberellin and excised immediately showed a sharp reduction in mite populations. Also, discs excised from plants immediately after they received the second of two sprays showed similar results.

Part of this variation in reproduction response from mites may be associated with nutrition. Data given in table 3 indicate that the size of the plants in Experiment B varied according to the amount and type of application of gibberellin treatment that they received. It has

Table 3.—Mean plant height and leaf measurements in inches following gibberellin treatment of plants used in experiment B.

TYPE OF TREATMENT	PLANT HEIGHT	LEAF LENGTH	LEAF WIDTH
None	11.0	4.5	3.25
One 3-second aerosol spray	28.0	5.5	3.5
Two 3-second aerosol sprays	30.5	5.5	3.75
Two 3-second aerosol sprays cut at half growth	15.5	5.0	3.5
Three 3-second aerosol sprays	30.0	5.75	3.75
Gibberellin slurry	16.5	5.0	3.5

been determined, however, that gibberellin treatment does not significantly change the total amount of major minerals in the plant, (Wittwer *et al.* 1957). If this is the case, a plant which receives the maximum stimulation from gibberellin and becomes twice as large as a control plant would have only one-half the concentration of mineral per unit volume. A mite feeding on this plant would receive only one-half the normal concentration of minerals. Since the reproductive capacity of mites is directly related to nutrition (Rodriguez 1951), this could result in a reduction in the population.

Factors other than the growth response of plants treated with gibberellin may also affect mite populations. This is indicated by the fact that there was a reduction of mite populations on discs cut from plants before they had time to show significant growth response. This population drop almost equalled the reduction of mites recorded on discs that had been excised from fully grown plants which had received three sprays of gibberellin.

It was noted in Experiment B that discs from control plants which had been infested with mites from control plants had fewer eggs than were recorded from comparable combinations in Experiment A. This difference may have been caused by an infection of powdery mildew which appeared on the plants before the discs were cut. It was observed that the leaves of the control plants seemed to be much more susceptible to mildew infection than were leaves of plants that received one or more applications of gibberellin.

The same observation was made on the discs after they had been cut. Although care was taken to cut discs from leaf parts without mildew infection, within 5 days the disease usually appeared on the control discs. Mite populations on the discs were not affected since the mildew did not appear until the fifth day after infestation.

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Control of the Lesser Cornstalk Borer Attacking Peanuts[1]

JAMES A. HARDING[2]

ABSTRACT

Results of field studies made during 1958 and 1959 for the purpose of developing effective control measures against the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell.), on peanuts grown under sprinkler irrigation are reported herein. Accumulated data show that granulated forms of insecticides are generally as effective as sprays in combating this insect. Endrin, SD-4402 (1,3,4,5,6,7,8,8-octochloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), parathion and DDT were most effective in reducing larval damage to the pegs and nuts. Residual effectiveness of these insecticides proved limited under irrigation conditions imposed in the tests. Effective control of the insect was also obtained when these insecticides were applied as sprays under pressures of 30 to 40 p.s.i. at the rate of 5 to 20 gallons per acre.

Studies on insect pests of peanuts in Texas were initiated in Erath County in 1954 and in Frio County in 1958, at the request of numerous growers. The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell.), has been identified as the insect possessing the greatest damage potential.

The tests reported here were conducted at Pearsall in Frio County, where the crop is grown under sprinkler irrigation. The acre value of the crop is relatively high which permits the grower to make expenditures to conduct disease and pest control programs that would not be economically feasible under dryland conditions.

Irrigation and the long growing season make possible the production of two crops per season. The "spring crop" is planted late in March and is harvested in early August. "Fall peanuts" are planted in late June or early July and are harvested in the latter part of October or early in November. Usually the lesser cornstalk borer constitutes a problem only on fall plantings. Injury to the young plants occurs about 2 weeks after emergence and continues until harvest.

Luginbill & Ainslie (1917) studied the lesser cornstalk borer and recommended winter cleanup of plant debris for control as did Isely & Miner (1944) working with this insect on beans. Kelsheimer (1955) and Wilson & Kelsheimer (1955) reported preventive control with 1.5 to 2 lbs. of actual chlordane per acre on cowpeas and stated that larvae present underground could not be contacted with an insecticide. Sanchez (1956) studied the seasonal life history of the insect in Texas. Reynolds *et*

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al. (1955) reported that lesser cornstalk borer infestations on sorghums originate from field residents rather than egg deposition. They recommended field cleanup several weeks before seeding, flood irrigation when a serious infestation is present, and preventive applications of endrin, aldrin, heptachlor, or dieldrin as sprays or granules at the time of plant emergence for control of migrating larvae. Cunningham *et al.* (1959) obtained control of the lesser cornstalk borer on peanuts with spray applications timed with the appearance of the moths of each generation. Effective results were obtained with DDT applied at 1.5 lb./A. and endrin at 0.4 lb. per acre. They found no differences in control that could be attributed to variations in spray volumes or interval between sprays.

The objectives of the tests reported here were to develop effective control measures for the lesser cornstalk borer attacking sprinkler irrigated peanuts by: determining the effect of variations in spray application dates on larval damage; comparing the effectiveness of various toxicants; and comparing granulated and spray formulations of insecticides.

MATERIALS AND METHODS.—Experiments were designed as randomized blocks in which each treatment was replicated 4 times. Plots varied from 2 rows to 6 rows wide and were 50 feet long in 1958 tests and 35 feet long in 1959 tests. Twenty-foot alleys were left between replicates to facilitate harvest. Spantex peanuts were planted in 36-inch rows on a slightly elevated bed.

Granules were applied by hand to each plot and sprays were applied with either a portable power sprayer or with a 3-gallon compressed air sprayer. Two nozzles per row were used to direct the spray toward the base of the plants. In the 1958 tests, 20 gal./A. of spray formulation were delivered at a pressure of 40 p.s.i. and in the 1959 tests, 5 gal./A. were applied at a pressure of 30 p.s.i.

Insect damage was evaluated at specified intervals by examining the pegs and nuts on 5 to 10 randomly selected plants from the outside rows in each plot. The percentage of damage to the pegs and nuts was calculated by dividing the total number of pegs and nuts into the number damaged. The middle rows in each plot were harvested with commercial threshers to obtain records on yield.

Table 1.—Effect of granulated insecticides applied approximately 30 days after planting on lesser cornstalk borer damage, 1958.

Toxicant	Pounds Actual per Acre	Per Cent Damage to Pegs and Nuts*	
		Days After Application	
		Test A, 31	Test B, 29
DDT	3	0.6[a]	1.0[a]
Di-Syston	1	0.7[a]	1.9[a]
Parathion	1	0.6[a]	0.8[a]
Phorate	1	0.6[a]	1.0[a]
Dieldrin	1	—	0.7[a]
Check	—	3.1[b]	2.2[a]

* Applying Duncan's Multiple Range Test, if two treatments do not have a common letter for a given column the treatments are significantly different at the 5% level of probability. Superiority follows the alphabet, in that *a* is superior to *b* and the letter farthest from *a* is the most inferior.

Table 2.—Effect of varying the date of spray application of a half pound of actual endrin per acre on lesser cornstalk borer damage.*

Date of Application (1959)	No. of Damaged Plants/20 (Aug. 10)	Per Cent Damaged Pegs and Nuts (Sept. 9)	No. of Pegs and Nuts (Sept. 9)	Per Cent Damaged Pegs and Nuts (Sept. 23)	No. of Pegs and Nuts (Sept. 23)
July 27	2.35[a]	14.8[a]	114.3[a]	18.0[a]	134.0[a]
Aug. 28	—	10.8[a]	122.8[a]	16.5[a]	181.0[a]
July 27 and Aug. 28	—	6.8[a]	144.3[a]	10.5[a]	145.3[a]
Check	16.25[b]	36.0[b]	82.8[b]	39.5[b]	150.3[a]

* Refer to footnote, table 1.

Yield data will not be presented because no differences occurred except in test B (table 1), where all treatments increased the yield over the check plots.

The chemicals included in these tests are DDT, endrin, dieldrin, aldrin, dimethoate, heptachlor, parathion, ronnel, toxaphene, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Perthane® (1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane), phorate, Di-Syston® (*O-O*-diethyl *S*-2-(ethylthio)-ethyl phosphorodithioate), and SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan).

RESULTS AND DISCUSSION.—Data obtained in a test designed to apply sprays coinciding with the first and second generation moth flights are summarized in table 2. On August 10, there were fewer damaged plants in treated plots than in the check. Records obtained on September 9, showed that the treatments significantly reduced the amount of damage and the number of pegs and nuts was increased. This demonstrates protection afforded the developing pegs before they were large enough to be included in damage records. Records obtained on September 23 showed a reduction in damage in treated plots but no significant increase in the number of pegs and nuts.

Results of similar tests designated as A and B are presented in table 1. Granulated insecticides were applied in a swath 1 foot wide directly over the plants approximately 30 days after planting. Although the infestation was light, analysis of test-A records showed that all treatments caused a highly significant reduction in damage. In test B, no differences in the extent of injury were found.

Table 3 summarizes the effect on lesser cornstalk borer injury of an application of granulated systemic insecticides 30 days after planting. Differences in damage were apparent 28 and 58 days after treatment but no differences were observed 77 days after treatment. Phorate proved the most effective chemical used for control of the lesser cornstalk borer.

In another test DDT, dieldrin, heptachlor, and toxaphen in granulated formulations were applied to peanuts at the time of planting and then tilled into the soil. The data shown in table 4 indicate that these chemicals were effective in controlling the pest 28 days after application but were no longer effective after 58 days. Dieldrin showed some superiority in control but all insecticides

Table 3.—Effect of granulated systemic insecticides applied at 2 lbs. actual per acre at time of planting on lesser cornstalk borer damage, 1959.

Toxicant	No. of Damaged Plants/20*	Per Cent Damage to Pegs and Nuts*	
	Days After Application		
	28	58	77
Phorate	12.3[a]	28.3[a]	29.5[a]
Di-Syston	9.8[a]	32.5[a,b]	29.8[a]
Dimethoate	15.0[b]	43.3[b]	33.8[a]
Check	18.5[c]	39.8[a,b]	35.8[a]

* Refer to footnote, table 1.

Table 4.—Effect of granulated insecticides applied at time of planting on lesser cornstalk borer damage, 1959.

Toxicant	Pounds Actual per Acre	No. of Damaged Plants/20*	Per Cent Damage to Pegs and Nuts*	
		Days After Application		
		28	58	77
DDT	2	13.5[a,b]	39.0[a]	43.3[a]
Dieldrin	1	11.5[a]	24.8[a]	40.8[a]
Heptachlor	1	14.0[b]	36.8[a]	40.5[a]
Toxaphene	3	14.5[b]	43.5[a]	47.0[a]
Check	—	18.5[c]	43.8[a]	44.5[a]

* Refer to footnote, table 1.

Table 5.—Effect of various insecticides applied as sprays, July 27, on lesser cornstalk borer damage, 1959.

Toxicant	Actual per Acre (Lb.)	No. of Damaged Plants/20*	Per Cent Damage to Pegs and Nuts*
		Days After Application	
		14	44
Perthane	1.00	5.3[a]	47.5[b,c]
Heptachlor	1.00	1.5[a]	36.0[a,b]
Toxaphene	1.50	4.5[a]	32.8[a,b]
Endrin	0.50	2.3[a]	19.3[a]
Ronnel	0.75	6.5[a]	46.3[b,c]
Thiodan	0.75	6.8[a]	34.3[a,b]
Guthion	0.50	5.0[a]	39.3[b]
DDT	1.00	5.8[a]	40.3[b]
Check	—	16.8[b]	57.8[c]

* Refer to footnote, table 1.

included in the test lacked residual effectiveness.

Data in table 5 are a summary of a test with various insecticides applied as sprays on July 27, 1959, before larvae could be found by screening the soil in the peanut field. Fourteen days after application all treatments reduced damage significantly and uniformly over the check. Forty-four days after treatment endrin was the most effective toxicant. Toxaphene, Thiodan, and heptachlor ranked next in effectiveness, Guthion and DDT were less effective. The injury in Perthane and ronnel-treated plots did not differ from that in the check.

Records presented in table 6 are from a test similar to

Table 6.—Effect of various insecticides applied as sprays, August 13, on lesser cornstalk borer damage, 1959.

Toxicant	Pounds Actual per Acre	Per Cent Damage to Pegs and Nuts*	
		Days After Application	
		27	41
Heptachlor	1.00	38.3[d]	30.0[b,c]
Endrin	0.5	6.5[a]	10.3[a]
Toxaphene	2.0	25.8[c]	19.3[a,b]
Parathion	0.5	7.3[a]	16.8[a]
Aldrin	1.0	37.8[d]	37.8[c]
DDT	1.0	11.3[a,b]	13.3[a]
Dimethoate	0.5	21.0[b,c]	35.5[c]
SD-4402	0.5	2.3[a]	7.0[a]
Check	—	38.0[d]	38.3[c]

* Refer to footnote, table 1.

Table 7.—Effect of two toxicants applied as high- and low-gallonage sprays and as granules on lesser cornstalk borer damage, 1958.

Toxicant	Type of Application	Pounds Actual per Acre	No. of Damaged Plants/80*		Per Cent Damage to Pegs and Nuts*	
			Days After Application			
			7	14	23	36
DDT	10 gal.	2	15[a]	32[a]	12.5[b]	5.9[b]
	80 gal.	2	25[a]	40[a]	11.3[b]	6.1[b]
	Granules	2	4[a]	42[a]	7.5[a,b]	4.4[a]
Parathion	10 gal.	1	17[a]	37[a]	4.4[a]	1.4[a]
	80 gal.	1	21[a]	45[a]	3.8[a]	3.1[a]
	Granules	1	9[a]	43[a]	2.1[a]	2.8[a]
Check	—	—	47[b]	74[b]	28.2[c]	5.8[b]

* Refer to footnote, table 1.

the previous one except that the materials were applied on August 13 when soil screening revealed many larvae in the second to fifth instars. Data recorded 27 days after treatment indicated that SD-4402, endrin, DDT, and parathion were most effective in reducing lesser cornstalk borer damage. Dimethoate and toxaphene ranked next in effectiveness. Injury in the heptachlor- and aldrin-treated plots did not differ from that in the check. Forty-one days after application damage records showed that SD-4402, endrin, DDT, and parathion were the most effective in reducing damage. Toxaphene was slightly less effective. Heptachlor, aldrin, and dimethoate were not effective.

Table 7 summarizes results of an experiment designed to determine the effect of insecticides applied as granulated materials, and sprays at varying gallonages. When this test was initiated, screening of the soil revealed many larvae in the second to fifth instars. Analysis of the data indicates that injury was less severe in treated plots than in the check 7 and 14 days after application. Twenty-three days after application all treatments reduced damage significantly. Parathion treatments were more effective than DDT sprays. Thirty-six days after application, plots treated with parathion and DDT granules were superior to those treated with DDT sprays and the untreated checks. Further counts were not obtained owing to a reduction in the infestation.

Data obtained in a test comparing the effectiveness of granules and sprays are illustrated in table 8. No differences appeared owing to the method of application

Table 8.—**Effect of granulated and spray formulations of insecticides on lesser cornstalk borer damage, 1959.**

Toxicant	Pounds Actual per Acre	Formulation	Per Cent Damage to Pegs and Nuts 21 Days After Application*
Endrin	0.5	Spray	14.3[a]
	0.5	Granules	20.0[a]
Parathion	1.0	Spray	13.7[a]
	1.0	Granules	19.2[a]
Check	—	—	35.2[a]

* Refer to footnote, table 1.

or to individual toxicants but all treatments reduced damage significantly.

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The Evaluation of Resistance of Seedling Alfalfa Varieties and Strains to the Spotted Alfalfa Aphid, *Therioaphis maculata*[1]

Wilfred J. Klement and N. M. Randolph[2]

Abstract

Four experiments were conducted outdoors during the spring and summer of 1959 to determine the degree of resistance of alfalfa plants to attacks of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). Of the varieties tested Cody, Lahontan and Moapa exhibited the highest degrees of aphid resistance. Zia was considered moderately resistant and African, Atlantic, Epley, Orestan, Original C-40 and Ranger varieties were slightly susceptible to aphid damage. In at least one experiment, the plant injury to each slightly susceptible variety was significantly less than that to the more susceptible varieties. The remaining varieties included in the tests were susceptible to attacks by the aphid, however, the degree of susceptibility of each variety varied somewhat in the different experiments.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), caused severe damage to alfalfa in Texas during 1955 and 1956, Randolph (1957). The selection of alfalfa varieties resistant to the spotted alfalfa aphid offers an economical method of control for this insect.

The possibility of developing varieties and strains of host plants resistant to insects has received much attention for several years. Damage to wheat caused by the hessian fly, *Phytophaga destructor* (Say), has been greatly reduced by the use of resistant wheat varieties in Kansas and other mid-western states. Blanchard & Dudley (1934) first reported resistance in alfalfa plants to the pea aphid, *Macrosiphum pisi* (Harris). In field tests conducted during 1934, Painter & Grandfield (1935) noted that only a few plants of the Ladak variety were heavily infested or severely damaged by the pea aphid.

Alfalfa varieties tested by entomologists in Arizona (Dahms 1958) and found to be resistant to the spotted alfalfa aphid were Bam (Iran), Lahontan, Moapa, Nevada Synthetic, Zia (New Mexico 16), and Sirsa 9 (India). Cody (Kansas Synthetic B_1) was added to this list by Painter (1958).

Harvey & Hackerott (1956) working in Kansas found that only 1 to 10 of 500 seedlings of each of the following varieties survived an infestation of the spotted alfalfa aphid: Baltic, Cossack, Grimm, Ladak, Meeker, Nomad and Ranger. Only 209 of 25,000 Buffalo seedling plants survived an infestation of the aphid. The percentage of Lahontan seedlings killed by the feeding of the aphid was very low.

Howe & Smith (1957) submitted the seedlings of Caliverde, African and Lahontan varieties of alfalfa to infestations of the spotted alfalfa aphid and reported that all of the Caliverde seedlings were killed within 2 weeks. Seedlings of the African variety exhibited tolerance to the aphid. However, almost all mature plants of the African variety were killed or injured when infested with the spotted alfalfa aphid. Lahontan alfalfa plants in seedling and mature stages possessed a high degree of resistance.

Cody, a new synthetic variety of alfalfa resistant to the spotted alfalfa aphid, was developed by screening more than 250,000 plants of Buffalo alfalfa (Painter 1958, 1959). The new variety resulted from synthetic combinations of 22 of the selected plants.

Methods and Materials.—Studies to determine the degree of susceptibility of seedling alfalfa plants to attacks of the spotted alfalfa aphid were conducted outdoors during the spring and summer of 1959 at College

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Station, Texas. The tests included four similar but independent experiments.

Different varieties of seedling alfalfa plants were subjected to attacks by the spotted alfalfa aphid. The seedlings used in the tests were grown in 1-gallon buckets which were kept on wooden tables enclosed within a plastic screen cage approximately 6′×6′×36′ in size. A hygrothermograph located inside the cage provided temperature and humidity records for the duration of each test period.

A stock culture of aphids was maintained on mature alfalfa plants growing in 1-gallon buckets enclosed in individual cages covered with plastic screen of fineness sufficient to prevent escape of the insects. Aphids for establishing the culture were obtained from alfalfa plants growing in the vicinity of College Station.

Experiments I, II, and IV involved the testing of five seedlings of each variety. Ten seedling alfalfa plants per gallon bucket were tested in Experiment III. Varietal plantings were randomized and replicated four times.

A 10″×10″×$\frac{1}{4}$″ plywood board with a 5$\frac{1}{2}$″ circular hole in the center was placed on the upper-rim of each gallon bucket in which the plants were growing. The plywood served as a platform for a cage 6″×6″×12″. The cage, constructed of a light wooden framework and covered with 32-mesh plastic screen, was placed over the plants. The top of the cage was made of wood and the bottom was open. Alfalfa seedlings were permitted to reach the one-true-leaf stage of growth before the introduction of aphids. Aphids from the stock culture were transferred to plants to be tested. One apterous adult or nymph aphid was placed on or near each alfalfa plant.

Records of aphid populations were taken at 3-day intervals and were obtained by counting the number of aphids on three randomly selected alfalfa leaflets on each replicated planting under test in Experiments I, II and IV. Aphid populations in Experiment III were obtained by counting the aphids on six randomly selected leaflets on the plants.

Plant injury was evaluated at 3-day intervals. The extent of injury to the plants resulting from the feeding activities of the aphids was rated on a scale from 1 to 9. Ratings of 1 and 9 represented no injury and death of the plant, respectively.

Plants of each alfalfa variety were arranged in a randomized block design and the data were subjected to an analysis of variance. Comparisons among means were tested for significance by Duncan's multiple range test at the 5% level.

Table 1.—Comparison of variety plant injury means, Experiment I.

VARIETY	MEANS OF PLANT INJURY[a]
Lahontan	2.93
Moapa	2.97
Zia	3.80
Ranger	5.36
African	5.83
Original C-40	5.87
Rhizoma	6.22
Atlantic	6.34
Epley	6.44
Rambler	6.45
Caliverde	6.51
Socheville	6.52
Buffalo	6.55
Chillicothe Common	6.65
Hairy Peruvian	6.94
Southwest Common	6.95
Teton	6.98
Arizona Chilean	7.13
Williamsburg	7.20
Indian	7.26
DuPuits	7.43
Nomad	7.57

[a] Means flanked by the same line are not significantly different at the 5% level.

Table 2.—Comparison of variety plant injury means, Experiment II.

VARIETY	MEANS OF PLANT INJURY[a]
Cody	1.82
Lahontan	1.92
Moapa	2.16
Zia	2.70
African	3.65
Ranger	4.08
Original C-40	4.29
Orestan	4.81
Socheville	5.30
Epley	5.49
DuPuits	5.57
Buffalo	5.62
Rambler	5.65
Atlantic	5.76
Williamsburg	6.06
Ladak	6.19
Sevelra	6.21
Cossack	6.29
Chillicothe Common	6.47
Teton	6.47
Hairy Peruvian	6.52
Grimm	6.64
Arizona Chilean	6.86
Indian	6.90
Rhizoma	6.94
Nomad	7.04
Southwest Common	7.31
Narragansett	7.48
Caliverde	7.62

[a] Means flanked by the same line are not significantly different at the 5% level.

RESULTS.—*Experiment I.*—Means of plant injury of 22 varieties indicated that Lahontan and Moapa possessed the highest degree of aphid resistance, table 1. These two varieties were significantly less susceptible to aphid injury than each of the other varieties included in the test. The variety Zia possessed aphid resistance of a significantly lower degree than Lahontan or Moapa, however, the degree of resistance was significantly higher than that of the remaining alfalfa varieties. Varieties African, Original C-40 and Ranger were moderately susceptible to injury by the aphid. The other entries varied in the degree of aphid susceptibility.

Experiment II.—A comparison of means of plant injury of 29 varieties showed that Cody, Lahontan and Moapa responded to aphid injury in a similar manner. These varieties exhibited the greatest aphid resistance and the amount of plant injury was significantly lower than that suffered by plants of all other varieties. The variety Zia demonstrated a smaller degree of aphid resistance than the seedlings of the Cody, Lahontan and

Moapa. African, Original C-40, and Ranger varieties were rated as moderately susceptible to injury by the aphid. The remaining varieties differed in the extent of susceptibility as shown in table 2.

Experiment III.—Means of plant injury of the 29 varieties included in this test were compared and the varieties Cody, Lahontan and Moapa were found to possess the greatest degree of aphid resistance, table 3. Plant injury in these varieties was significantly less than that of each of the remaining varieties. Zia proved less resistant to the spotted alfalfa aphid attacks than varieties Cody, Lahontan or Moapa. Seedling plants of the varieties Epley and African were but moderately susceptible to attacks by the aphids.

Experiment IV.—A comparison of the 27 variety means for plant injury indicate that seedlings of Lahontan and Moapa varieties responded similarly and had the highest degrees of resistance to the aphid, table 4. The variety Cody exhibited high resistance to the spotted alfalfa aphid, however, the degree of resistance was significantly smaller than that shown by Lahontan or Moapa. The aphids caused greater damage to plants of the variety Zia than to those of Cody. Seedlings of varieties African, Atlantic, Epley and Orestan were moderately susceptible to the feeding of the aphid. The remaining varieties varied in degrees of susceptibility to the aphid.

Table 3.—Comparison of variety plant injury means, Experiment III.

VARIETY	MEANS OF PLANT INJURY[a]
Moapa	2.00
Lahontan	2.16
Cody	2.46
Zia	3.00
Epley	5.26
African	5.38
Orestan	6.10
Ranger	6.25
Original C-40	6.26
Caliverde	6.34
Buffalo	6.47
Hairy Peruvian	6.52
Cossack	6.59
Indian	6.66
Rambler	6.70
Narragansett	6.78
Sevelra	6.79
Socheville	6.85
Atlantic	6.92
Nomad	7.05
Arizona Chilean	7.09
Rhizoma	7.10
Southwest Common	7.12
Teton	7.17
Ladak	7.30
Williamsburg	7.32
Grimm	7.58
DuPuits	7.89
Chillicothe Common	7.91

[a] Means flanked by the same line are not significantly different at the 5% level.

Table 4.—Comparison of variety plant injury means, Experiment IV.

VARIETY	MEANS OF PLANT INJURY[a]
Lahontan	1.96
Moapa	1.97
Cody	2.57
Zia	3.13
Atlantic	4.72
Epley	4.74
Orestan	4.82
African	5.05
Rambler	5.41
Buffalo	5.43
Socheville	5.58
Chillicothe Common	5.59
Ladak	5.63
Cossack	5.65
Teton	5.79
DuPuits	5.79
Williamsburg	5.80
Caliverde	5.95
Arizona Chilean	5.95
Hairy Peruvian	5.97
Rhizoma	6.02
Ranger	6.12
Indian	6.15
Barstow Common	6.31
Nomad	6.32
Grimm	6.37
Southwest Common	6.42

[a] Means flanked by the same line are not significantly different at the 5% level.

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Control of the Corn Root Webworm[1]

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Abstract

In the lower Piedmont region of Virginia the corn root webworm, *Crambus caliginosellus* Clem., was found to be an important pest of seedling corn growing on eroded Cecil sandy loam soil following a depleted stand of lespedeza with heavy weed growth.

Experiments for control of this insect were conducted in 1955 and 1957 with aldrin, chlordane, and heptachlor applied both in the furrow and broadcast; and with dieldrin as a seed treatment. Granulated aldrin, chlordane, and heptachlor applied in the furrow at the rate of 1.0 pound per acre were equally effective in preventing corn root webworm damage. In the treated plots the yield was increased approximately 50%. Less damage resulted when the aldrin- and heptachlor-fertilizer mixtures were used at the rate of 1.0 pound per acre, as compared to 0.5 pound per acre; but the yield data from the two rates of application were not significantly different. Plants in the plots where dieldrin was employed as a seed treatment showed more injury than those in which aldrin- or heptachlor-fertilizer mixtures were used at the 0.5 pound dosage, but the differences were not significant. Poor results were obtained in a test in which aldrin- and heptachlor-fertilizer mixtures were placed in a narrow area to the side of the seed with tractor-mounted equipment during the planting operation.

A preplanting treatment with granulated aldrin or heptachlor, applied broadcast at 1.8 to 2.0 pounds per acre, gave excellent corn root webworm control.

For many years the corn root webworm, *Crambus caliginosellus* Clem., was an important insect pest of newly set tobacco in Virginia, but it is of little significance at the present time. Scott (1955) reported that injury to tobacco in Tennessee by this insect had not been noted since 1943 and suggested that the change in its status seemed to be linked with changes in agricultural practices. More recently McEwen & Hervey (1958) reported economic damage by the corn root webworm to carrots in western New York State.

During some years the corn root webworm is an important insect attacking seedling corn in the lower Virginia Piedmont. In 1957, damage in many areas of Pittsylvania county was extensive and some plantings were abandoned, or the land planted to other crops. Infestations were found to be common on eroded Cecil sandy loam soil planted to corn following a depleted stand of lespedeza, *Lespedeza stipulacea* Maxim., which contained a heavy growth of weeds. Runner (1914) listed buckhorn plantain, *Plantago lancealata* L., and the oxeye daisy, *Chrysanthemum leucanthemum* L., as the preferred weed host plants of the corn root webworm in Virginia. Ainslie (1922) reported that the small larvae pass the winter around the roots of such weeds as stickweed, *Aster dumosus* L., fleabane, *Erigeron* spp., and buckhorn plantain. The white heath aster, *Aster ericoides* L., was reported to be an important weed host in Tennessee (Scott 1955). In the Virginia Piedmont, all of the named weeds are common to fields planted to corn in which the most damaging infestations occur.

Injury.—Corn root webworm damage is noted soon after the seedlings appear above ground. The leaves which are distorted tend to stand out from the stem, and often the middle leaf is severed at the base, thus killing the bud. The larvae feed on the corn plant mostly below the soil surface, usually killing young plants and severely affecting others which send out suckers that rarely produce grain. In 1957, a total of 13 larvae were taken from the soil around a recently killed corn plant in a severely infested field. Injury was more severe in corn fields planted later than the normal planting date and during which little rainfall occurred for a period of 2 or more weeks after planting.

Control.—Ainslie (1922) suggested control by employing cultural practices such as rotations, and plowing land in July or August that is intended to be planted to corn the following year. Other recommended practices included proper seed bed preparation, adequate fertilization, good seed, thick stands, and early planting.

Workers in a number of states have conducted research during the past decade on the control of various subterranean insects which attack field corn by employing some of the chlorinated hydrocarbon insecticides as soil and seed treatments (Apple *et al.* 1958, Kulash & Monroe 1955, Cox & Lilly 1953). A preliminary experiment conducted at Chatham, Virginia, in 1955 indicated that aldrin, chlordane, and heptachlor were promising insecticides for the control of the corn root webworm. Further tests in 1957, using aldrin and heptachlor applied in the furrow and broadcast, substantiated these results. Dieldrin as a seed treatment was also included in one test.

The efficiency of the insecticides was determined by counting the stand, the number of plants killed or damaged by the insect, and collecting yield data. Statistical treatment of the results included the multiple range test (Duncan 1955).

Furrow Treatment.—In the preliminary experiment, aldrin, chlordane, and heptachlor were applied as granules in the furrow on the appropriate planting date. Some mixing of the insecticides with the soil was accomplished by the use of a small plow. The soil type was an eroded Cecil sandy loam on which the previous cover had consisted of weeds for a period of several years. The plots were single row, 65 feet long, and each treatment was replicated five times in randomized blocks. The soil was dry on the planting date and little rainfall occurred for a period of 3 weeks thereafter. A moderate to severe infestation of the corn root webworm developed in the test area.

The results are presented in table 1. Final stand counts on May 25 showed similar results in the treated plots, but

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Table 1.—Corn root webworm control with insecticides applied in the furrow, broadcast, and as a seed treatment.

TREATMENT	TOXICANT PER ACRE (LB.)	PLANT STAND	NO. OF PLANTS KILLED OR DAMAGED BY WEBWORMS[a]	YIELD (BU./ ACRE)[a]
1955, Furrow				
Granulated				
Aldrin	1.0	63	0.8	69.3
Heptachlor	1.0	66	1.0	71.1
Chlordane	1.0	63	1.2	65.4
Check	—	31	32.4	33.5
1957, Experiment 1, Furrow				
Fertilizer mixtures				
Aldrin	1.0	134	2.0	74.5
Heptachlor	1.0	138	4.5	73.8
Aldrin	0.5	132	13.8	69.6
Heptachlor	0.5	133	14.5	67.3
Seed treatment				
Dieldrin 50%, WP[b]	—	100	20.2	62.7
Check	—	124	32.2	62.6
1957, Experiment 2, Furrow				
Fertilizer mixtures[c]				
Aldrin	1.25	50	9.5	—
Heptachlor	1.25	45	10.5	—
Check	—	32	24.8	—
1957, Experiment 3, Broadcast				
Granulated				
Aldrin	2.0	65	0.0	46.1
Heptachlor	2.0	68	0.3	43.6
Check	—	53	14.8	36.1
1957, Experiment 4, Broadcast				
Granulated				
Aldrin	1.8	134	1.5	—
Heptachlor	1.8	145	1.7	—
Check	—	95	58.5	—

[a] Means paralleled by same vertical line are not significantly different at the 5% level.
[b] Two ounces per bushel of seed.
[c] Applied with tractor-mounted equipment; insecticide-fertilizer mixtures placed in an area to the side of the seed.

a high reduction in the plant stand was present in the check. The three insecticides were equally effective in reducing corn root webworm damage, and the yield ranged from 65.4 bushels per acre for the chlordane to 71.1 bushels for the heptachlor treatment. The differences in the yield between the three insecticide treatments were not significant, but were superior to the check in which the yield was reduced approximately one-half.

In experiment 1 of the 1957 tests, aldrin- and heptachlor-fertilizer mixtures at two dosages were compared; and dieldrin was included as a seed treatment. The plots were single row, 110 feet long, and were replicated six times in randomized blocks. The test area was located on Cecil sandy loam soil and the previous plant cover had consisted of a depleted stand of red clover with moderate weed growth. The fertilizer-insecticide mixtures were applied to the bottom and sides of the furrow, and some mixing was accomplished by the mechanical planter.

The results given in table 1 show little differences in the plant population between the four insecticide-fertilizer mixtures and the check, but the plant stand in the dieldrin seed treatment was poorer. Aldrin in the fertilizer at 1.0 pound per acre was more effective in preventing corn root webworm damage than aldrin or heptachlor at the 0.5 pound dosage. The difference between heptachlor at the 1.0 pound per acre dosage and the two insecticides at 0.5 pound was not significant. Aldrin and heptachlor at the 0.5 pound per acre dosage and the dieldrin seed treatment were not significantly different, but the three treatments were superior to the check. The inferior results obtained with the seed treatments were also substantiated in a field in which the grower used lindane for coating the seed. The plant stand in this field was greatly reduced by the corn root webworm. The yield in the insecticide-fertilizer mixture treatments ranged from 67.3 to 74.5 bushels per acre; however, these differences were not significant. The yield in plots treated with aldrin- and heptachlor-fertilizer mixtures at the 1.0 pound per acre dosage was superior to the dieldrin seed treatment and to the check.

In experiment 2, aldrin- and heptachlor-fertilizer mixtures were applied with a tractor-mounted fertilizer applicator and planter which placed the fertilizer in an area to the side of the seed. The plots were six rows wide and 130 feet long, and were replicated twice in randomized blocks. The results were determined by counting the killed and damaged plants in 50 feet of row space on the two center rows of each plot. Aldrin- and heptachlor-fertilizer mixtures applied by the method described above gave similar results, but the control obtained was not considered satisfactory since the reduction in the corn root webworm damage was only about 58% (table 1). A total of six larvae was found in the vicinity of a severely injured plant on the side where there was no insecticide-fertilizer mixture.

BROADCAST SOIL TREATMENT.—Two experiments were conducted in 1957 in which aldrin and heptachlor as granules were applied as preplanting broadcast treatments. After the materials were applied, they were immediately disked into the upper 2 to 3 inches of soil. Each experiment was located at a separate location on eroded Cecil sandy loam soil, and the previous plant cover had been a depleted stand of lespedeza and weeds, consisting largely of plantain and fleabane.

In experiment 3, each plot was six rows wide by 55 feet long; and each treatment was replicated four times in randomized blocks. Plant stand counts, injury data, and yield records were taken from the two center rows of each plot. Stand counts on May 21 were similar for both aldrin and heptachlor, but a slight reduction occurred in the check (table 1). Injury counts indicated similar reduction in corn root webworm damage for the two materials, and both treatments were superior to the check. The yield was affected by prolonged dry weather which prevailed during the silking period. An increase in yield of approximately 10 bushels per acre for the aldrin and 7 bushels for the heptachlor treatment was gained over the check; however, this difference was not significant.

Plots 14 rows wide by 244 feet long were used in experiment 4. Each treatment was replicated twice in randomized blocks. The granulated insecticides were applied with a combination grain-fertilizer drill 10 days before the seeding date.

The efficiency of the materials was determined on three

marked rows of each plot by counting the plant stand and the number of killed and injured plants in 150 feet of row space on each of three rows. Under the conditions of the experiment, aldrin and heptachlor gave excellent corn root webworm control (table 1). On May 21, the plant stand counts showed similar plant populations for the two insecticide treatments; but the plant stand in the check was reduced about 29%.

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Metabolism of P^{32}-Delnav in Cattle[1]

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ABSTRACT

Radiolabeled Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)) was applied to steers dermally and orally. Analyses of blood, urine, and feces indicated that the insecticide was rapidly metabolized and excreted. After 7 days both animals showed only trace amounts of radioactivity in the urine and feces, but the elimination of 20.4% of the dermal treatment indicates efficient absorption of the insecticide in comparison with other organophosphorus insecticides. The presence of three major hydrolysis products in the urine of both animals indicates a cleavage of Delnav at the P–S+C as well as the P–S—C bond, with oxidation before or after hydrolysis.

In studies at Kerrville, Texas, Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)) was found to be effective against the winter tick (*Dermacentor albipictus* (Pack.)) (Drummond *et al.* 1959) and the horn fly (*Siphona irritans* (L.)) (Roberts 1959). The metabolism of this organophosphorus compound, also known as Hercules AC-528, in rats and cockroaches has been reported by Arthur & Casida (1959). The present paper reports work on the absorption, excretion, and metabolic fate of P^{32}-labeled Delnav in cattle treated dermally and orally.

METHODS AND MATERIALS.— The principal components of the technical radioactive Delnav[2] were 42% of the trans isomer and 16% of the cis isomer of 2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate). In addition, there was present 6% of the dioxene derivative (2-*p*-dioxenethiol *S*-(*O,O*-diethyl phosphorodithioate)) and 36% of unknown radioactive materials. The technical Delnav was formulated as a 30% emulsifiable concentrate with 10% Cooper Emulsifier DLV6 and 60% solvent[3] and diluted with nonradioactive emulsifiable concentrate having the same composition so as to facilitate application. The specific activity of the diluted emulsifiable concentrate was 110 c.p.m./μg. and that of the technical Delnav, 366 c.p.m./μg.

Two Hereford steers were treated with the P^{32}-labeled Delnav, one dermally and the other orally. The first animal, weighing 220 kg., was treated dermally with an emulsion prepared from 12 grams of the emulsifiable concentrate by diluting to 454 ml. with water. The emulsion was then divided equally into two chromatographic spray bottles and applied to the animal with pressurized carbon dioxide. Each bottle of material was used to treat half the animal from the shoulder to the rump. Misting from the bottles was prevented by holding the orifice within ½ inch of the hair. The application rate based on technical Delnav was 16.2 mg./kg.

During and after treatment the animal was confined in a cage so that its head projected through an opening in a wall into another room. A sheet of upholstery plastic lined the opening and was held closely about the neck by elastic. This method prevented contamination of the forward area and assured that any radioactivity within the animal could occur only through dermal absorption.

The second steer, weighing 243 kg., was treated orally with material obtained by column purification of the emulsifiable concentrate. A glass column 18 mm. in diameter was packed to a height of 10 cm. with purified alumina (aniontropic) mixed with water (2% by weight). The concentrate was applied in 2-gram aliquots to the top of the column and washed in with 50 ml. of Skelly B Solvent. Another 50 ml. of Skelly B was run through the column followed by 100 ml. each of distilled benzene and 99% methanol to isolate any additional phosphorus-containing materials.

Paper chromatography indicated that the purified radioactive fraction in the benzene eluent contained 87.6% of technical Delnav, including 62.8% of the cis and trans isomers and 24.8% of the dioxene derivative. The observable specific activity of the purified Delnav was 284 c.p.m./μg. The nonDelnav fraction consisted chiefly of diethyl phosphorothioic and phosphorodithioic acids. The Skelly B fraction contained no radioactive material, but the methanol fraction contained at least five materials, the major component of which chromatographed as diethyl phosphorodithioic acid.

[1] Accepted for publication February 24, 1960.

[2] Determined by Hercules Powder Co., Wilmington, Delaware.

[3] William Cooper & Nephews, Inc., Chicago, Illinois.

The purified radioactive material was encapsulated with fractured wheat and administered to the steer by means of a balling gun. The rate of application was 4.65 mg./kg., based on technical Delnav. The animal was confined in stanchions during the experimental period.

Following application of the radioactive material, samples of blood, urine, and feces were collected at regular intervals from both animals. Blood and urine were collected by methods previously described (Robbins *et al.* 1956, Kaplanis *et al.* 1959). Feces were taken from the central portion of the deposits as they occurred. Daily samples of hair from the sprayed animal were taken throughout the experimental period from five locations on the treated area.

Assays for total radioactivity in the blood, urine, feces, and hair samples were made with a Geiger-Müller tube. Samples of blood and urine were plated directly into cupped nickel planchets lined with tissue paper and air-dried at room temperature. Fecal samples were mixed thoroughly and 1-gram (wet weight) portions weighed out directly into the planchets. The hair samples were prepared for determination of total radioactivity by wet digestion with nitric acid and clarification with hydrogen peroxide. All counts made on plated samples were corrected for radioactive decay and absorption where applicable.

Extraction of the blood was accomplished using an equal volume of chloroform. Efficiency was 81% of the organosoluble material. Feces were extracted by adding an equal weight of water and 100 ml. of chloroform to 25-gram samples, mixed in a Waring Blendor, and centrifuged. The chloroform layer was removed and the residue re-extracted with acetone. The acetone and chloroform extracts (the organosoluble fraction) were then combined for determination of total radioactivity. Efficiency was 86% of the possible chloroform-acetone-soluble material.

The separation of Delnav and possible metabolites was accomplished by two paper chromatographic systems. (a) Salt separation method: Whatman No. 1 filter paper strips coated with 5% (V/V) Dow Corning 550 fluid[4] in acetone were developed with acetonitrile-water (85:15). (b) Delnav-component separation method: Whatman No. 1 paper coated with 5% (V/V) Dow Corning 550 fluid in acetone was developed in acetonitrile-water (35:65). The ascending technique was used for both systems.

The Delnav and metabolites were determined both radiometrically and colorimetrically. The developed chromatograms were cut into consecutive centimeter sections and radioassayed with a Geiger-Müller tube, a strip scanner, or by autoradiography with Kodak Blue Brand X-ray film. The Delnav components and the diethyl phosphorodithioic and phosphorothioic acids were detected by spraying the strips with 0.5% 2,6 dibromo-*N*-chloro-*p*-quinoneimine dissolved in cyclohexane (Menn *et al.* 1957). The diethyl phosphoric acid was detected with the Hanes-Isherwood reagent (Hanes & Isherwood 1949). The principal metabolic products and their Rf values are shown in table 1.

Table 1.—Rf values for Delnav and its major metabolites separated by two acetonitrile-water solvent systems.

COMPOUND	85:15	35:65
Cis-trans Delnav	0.85	0.033
Dioxene Delnav	.85	.24
Diethyl phosphoric acid	.15	.78
Diethyl phosphorothioic acid	.34	.78
Diethyl phosphorodithioic acid	.52	.78
Phosphoric acid	0	—

Table 2.—Radioactivity and inhibition of cholinesterase in the blood of animals at different times after dermal and oral treatment with P32-Delnav.

HOURS	TOTAL RADIOACTIVITY (μG./EQUIV./ML.)		CHOLINESTERASE INHIBITION (%)	
	Dermal	Oral	Dermal	Oral
1	—	0.155	—	10
3	0.477	.264	0	24
6	.474	.341	3	24
9	—	.388	—	24
12	.318	.411	10	41
18	.249	.365	0	66
24	.240	.250	16	83
36	.195	.155	12	53
48	.165	.116	—	48
72	.120	.085	19	69
96	.090	.053	16	43
120	.084	.041	3	38
144	.075	—	32	—
168	.030[a]	—	0	—

[a] Standard error greater than ±5%.

Cholinesterase determinations were made by the electrometric method of Michel (1949).[5]

RESULTS.—*Blood.*—When applied dermally, Delnav was absorbed into the blood very rapidly (table 2). The highest level of radioactivity occurred in the 3-hour sample, whereas in the orally treated animal it was at 12 hours. The loss of radioactivity following the peak concentrations was gradual in both animals, but somewhat faster in the orally treated animal even though its peak occurred later. One week (168 hours) after application, blood radioactivity in both animals was negligible.

Chloroform extraction of a combined 3- and 6-hour sample of blood from the dermally treated animal showed that only 2.61% was organosoluble material, presumably Delnav or closely related compounds. The 12-hour sample of blood from the orally treated animal yielded 3.06% of the total radioactivity as organosoluble material, again presumably Delnav or closely related compounds.

Delnav is apparently broken down very rapidly to nontoxic compounds by the blood system of cattle. This rapid breakdown may help to explain why it is not a very good systemic insecticide (Drummond 1958).

The effect of Delnav on cholinesterase inhibition was difficult to ascertain. In the dermal treatment there was no consistent pattern of inhibition, and the maximum was 32%. In the oral treatment the maximum inhibition was 83% and occurred at 24 hours. The cause of the difference between treatments, when the total and organosoluble fractions were similar, is not known.

Urine.—The radioactivity found in the urine of both animals followed a pattern similar to that of the blood.

[4] Dow Chemical Company, Midland, Michigan.

[5] Determined by Animal Disease and Parasite Research Division workers under the supervision of R. D. Radeleff.

Table 3.—Radioactivity concentrations in urine of animals after dermal and oral treatment with P^{32}-Delnav.

Hours	Total Radioactivity (μg. Equiv./Ml.)		Cumulative Per Cent of Administered Dose	
	Dermal	Oral	Dermal	Oral
1	—	90.8	—	0.7
3	139.0	202.5	0.9	4.1
6	226.9	214.9	2.7	10.3
9	—	219.6	—	16.8
12	180.6	141.2	5.1	22.6
18	114.4	83.0	6.5	32.2
24	87.4	84.6	8.2	40.3
36	77.5	77.6	10.5	51.8
48	44.7	32.9	12.3	58.9
72	21.9	22.5	14.9	65.4
96	21.9	10.9	16.8	68.3
120	23.6	5.7	18.4	69.9
144	12.6	2.9	19.5	70.2
168	11.7	—	20.4	—

The peak occurred fairly early in the 6-hour sample from the dermally treated animal and in the 9-hour sample from the animal treated orally (table 3). At the end of 1 week the dermally treated animal was still eliminating radioactive material in the urine at 5.2% of the maximum rate. A much higher percentage of the applied dose was eliminated by the orally treated animal. The elimination of 20.4% of a dermal application was higher than with some other organophosphorus insecticides, such as Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) where only 3% to 5% was eliminated (Kaplanis *et al.* 1959, Robbins *et al.* 1959).

The excretory patterns of the metabolites are indicated in table 4. The 85:15 acetonitrile-water paper chromatographic system showed that diethyl phosphoric, diethyl phosphorothioic, and diethyl phosphorodithioic acids were the major hydrolysis products. The presence of these materials was also confirmed by the autoradiography technique.

The 35:65 acetonitrile-water chromatographic system showed the presence of minute amounts of cis and trans Delnav, dioxene derivative, and three other unknown materials. One of the unknowns was present only in the urine of the dermally treated animal, which indicates that it probably originated as an impurity in the technical Delnav. A highly polar unknown (Unknown A) was present at the point of application of the urine and it is assumed to be phosphoric acid and/or material bound by urinary solids deposited on drying of the urine.

The percentages of each metabolite found were similar when determined by both the Geiger-Müller tube or the strip scanner.

The major pattern of excretion appears to be the result of the conversion of Delnav to diethyl phosphorodithioic and phosphorothioic acids and their conversion to diethyl phosphoric acid. This implies a split of the Delnav at the P−S≠C as well as the P≠S−C bond with oxidation prior to or after hydrolysis (Arthur & Casida 1959). Thus, for the orally treated animal the percentage of phosphorothioic acid decreased from about 90% at 1 hour to about 40% at 72 hours, whereas the percentage of diethyl phosphoric acid increased from 0.1% to about 50% during the same interval. A similar pattern is seen for the dermally treated animal, except that there was initially more diethyl phosphorodithioic acid. A possible explanation could be the presence of a high amount of the phosphorodithioic acid in the technical Delnav as determined by column chromatography. Analysis of the methanol extract showed that over 30% was diethyl phosphorodithioic acid.

Feces.—A greater percentage of the insecticide was eliminated in the feces of the orally treated animal than in that from the animal treated dermally (table 5). The feces of the dermally treated animal were not weighed; however, based on the ratio of the μg. equivalents per

Table 4.—Percentages of metabolic products in the urine of animals treated with P^{32}-Delnav, determined by 85:15 acetonitrile-water solvent.

Hours	Diethyl Phosphoric Acid		Diethyl Phosphorothioic Acid		Diethyl Phosphorodithioic Acid		Unknown A		Trace Materials[a]	
	Geiger	Scanner	Geiger	Scanner	Geiger	Scanner	Geiger	Scanner	Geiger	Scanner
Oral Treatment										
1	0.1	0	96.5	82.1	2.6	17.9	0.7	0	0	0
3	6.4	8.0	61.7	61.2	27.1	26.3	.4	0	0	4.5
6	12.9	14.2	56.1	57.7	26.8	24.7	.6	0	0	3.4
12	20.0	20.0	49.9	56.3	18.5	18.1	.3	0	0	5.6
24	27.0	25.2	37.8	62.0	5.9	7.0	1.8	0	0	5.8
48	33.0	35.0	40.0	48.6	15.0	16.4	2.6	0	0	0
72	41.8	52.4	39.6	42.9	14.5	4.8	1.2	0	0	0
96	47.9	59.3	39.0	40.7	12.0	0	1.0	0	0	0
Dermal Treatment										
3	5.8	5.0	49.5	46.8	37.0	45.4	2.8	2.7	0	0
6	13.7	11.5	51.0	48.5	28.6	29.9	3.1	2.6	0	7.6
12	20.8	15.9	47.7	52.8	22.4	29.0	3.5	1.0	0	1.3
24	30.0	27.2	45.0	47.2	21.0	20.4	1.0	0.5	0	11.8
48	36.3	38.5	41.6	47.9	10.1	6.6	1.6	0	0	7.0
96	30.7	27.0	42.0	57.0	14.9	16.0	1.0	0	0	0
144	43.9	56.0	33.9	38.0	10.2	6.0	0	0	0	0

[a] Diethyl phosphorodithioic acid, dioxene derivative, trans-cis Delnav, and unknowns.

Table 5.—Radioactivity in feces of the animals after oral and dermal treatment with P^{32}-Delnav.

	Oral			Dermal
Hours	Total Radioactivity (μg. Equiv./Gm.)	Per Cent of Administered Dose	μg. Equiv. Organo-soluble	Total Radioactivity (μg. Equiv./Gm.)
6	0	—	—	0.09
9	2.2	0.057	—	—
12	14.5	.780	5.7	—
18	16.5	2.80	6.4	4.40
24	10.5	3.20	4.1	1.30
36	9.0	3.40	1.3	—
48	5.2	1.30	—	.69
72	2.4	.81	—	—
96	.95	.54	—	.46
120	.42	.19	—	—
168	—	—	—	.24
Total		13.08		

gram figures for the two animals, the total amount would be less. The peak level of radioactivity in both animals appeared at 18 hours. A chloroform-acetone extract of the 18-hour feces from the orally treated animal yielded 39% of the total radioactivity. The radioactivity was nearly four times that found in the feces of the dermally treated animal. The extraction of the dermal feces yielded 32% of the total radioactivity.

Fat.—Residue analysis of omental fat from the dermally treated animal after 7 days indicated 1.54 p.p.m. Delnav equivalent (Claborn *et al.* unpublished data). Chromatographic analysis of the residue showed that 97.4% was technical Delnav, with 76.3% trans or cis Delnav.

Hair.—Samples of hair taken from the dermally treated animal showed 19,500 p.p.m. of technical Delnav immediately after treatment and 15,390 p.p.m. after 7 days. Thus, there was a 21% loss during the experimental period. None of the material on the hair changed to more polar components as indicated by chloroform to water partitioning.

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Effects of Light, Temperature, and Relative Humidity on the Diel Behavior of the Alfalfa Weevil, *Hypera postica*[1]

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Abstract

Observations made in 1958 showed that adults of the alfalfa weevil, *Hypera postica* (Gyll.), were more active during the night than during the day. In 1959, replicated sweep samples were taken at regular intervals over 24-hour periods and periods in early morning, to study the effect of light, temperature, and relative humidity on the diel behaviour of the adult weevils. The results strongly suggested that the diel behavior of the adults was related to light intensity, whereas larvae showed no response to either light, temperature, or relative humidity over the 24-hour period.

The alfalfa weevil, *Hypera postica* (Gyll.), was first discovered in New York in 1955 (Neunzig *et al.* 1955) and studies of its biology were begun in Dutchess County in 1958. During that summer, the observation was made, which had been recorded earlier (Parks 1913, Titus 1910), that the adult weevils were much more active at night than during the day. For example, in one alfalfa field on July 11 at 3 P.M., 9 adults were collected in 200 sweeps with a 15-inch diameter collecting net, as compared with 831 adults collected in the same number of sweeps in the same part of the field at 10 P.M. that night.

Experiments were initiated in 1959 to check the validity of these observations and to determine if light, temperature, or relative humidity showed any relationship to this diel behavior of the adults. In addition, larvae were also studied to determine if they too showed any marked differences in behavior over the 24-hour period.

Experimental Procedure and Results.—Field experiments were conducted wherein regular periodic

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[2] The authors acknowledge the assistance of Donald Adams who aided in the completion of these experiments.

Table 1.—Recordings of temperature, relative humidity, and light intensity in an alfalfa field over a 24-hour period. Dutchess County, New York, June 30 to July 1, 1959.

TIME	LIGHT INTENSITY (FOOT-CANDLES)	TEMPERATURE (° F.)	RELATIVE HUMIDITY (%)
12 noon	21,360.00	101	48
2 P.M.	19,580.00	102	46
4	19,580.00	92	50
6	11,570.00	83	65
7	6,230.00	75	86
8	672.00	66	97
9	1.86	62	97
10	0.39	60	97
11	0.33	59	97
12	—[a]	56	97
1 A.M.	—[a]	55	97
2	—[a]	55	97
3	—[a]	54	97
4	0.27	55	97
5	4.81	56	97
5:30	63.00	55	97
6	196.00	56	97
8	2,676.00	61	88
10	13,350.00	74	56

[a] Light intensity too low to be measured.

sweeps were taken either over a 24-hour period or during the early morning hours. In all experiments, the randomized complete block design was used. Four to six blocks, depending on the size of the population of weevils, were staked out, with each block containing from 13 to 19 plots, depending on the number of times sweep samples were to be taken over the test period. At regular intervals, a definite number of sweeps was taken from one plot within each block. The predetermined times for sweeping each plot were randomized among the plots in each block. During the course of the studies, temperature and relative humidity were recorded with a hygrothermograph[3] and light intensity was measured with an exposure meter.[4] One experiment was conducted in late June when the adult population was at its peak. Four blocks, each containing 19 plots (90′×9′), were staked out in a heavily infested alfalfa field. At 19 different times over the 24-hour period from 12 noon on June 30 to 10 A.M. on July 1, 30 sweeps were taken in 1 plot from each block.

Table 1 shows the recordings of light, temperature, and relative humidity at the various times over the 24-hour period. The temperature remained high until late afternoon, then began dropping, with a corresponding rise in relative humidity, and finally reached its lowest point in the early morning hours.

Light intensity and temperature were found to be closely correlated, except for a short period in early morning when light intensity increased while temperature and relative humidity often remained practically constant. This early morning period is important since it is possible to conduct an experiment in the field during this time interval with temperature and relative humidity remaining nearly constant, while light intensity increases.

A large increase in adult activity occurred in the evening between the hours of 7 to 9 P.M. (fig. 1). The numbers of adults collected during the night remained fairly constant, and an analysis of variance of the numbers found from 9 P.M. to 5 A.M. showed that there were no significant differences among them. However, a significant difference was detected between those numbers found during the night and the numbers found during the day.

There was some variation among the numbers of larvae collected during the 24-hour period. An analysis of variance, together with Tukey's H.S.D. test showed that significant differences in larval numbers existed between several of the sampling intervals. In view of the irregular intervals at which these differences occurred, the data, as a whole, were not indicative of any diel behavior of the larvae.

It will be noted that adult numbers decreased in the morning between the hours of 4 to 6 A.M. Table 1 shows that between those hours, temperature varied only 1 degree, relative humidity remained constant, but light intensity increased from 0.27 to 196 foot-candles. These data suggest that light is the factor that affects the diel behavior of the adult weevils. Similar results also were found in preliminary experiments conducted during 1958 and 1959.

Another experiment was conducted to determine the relationship of temperature, relative humidity, and light intensity on adult and larval populations over a 2.5-hour period in early morning. A time interval was selected that would include the period when the adults changed their habits from night time activity to daytime repose.

At nine different time intervals over the period from 4:30 A.M. to 7:00 A.M. on August 1, 1959, 50 sweeps were taken from one plot in each of six blocks which had been staked out in a new heavily infested alfalfa field. Table 2 shows the recordings of temperature, relative humidity, and light intensity over the 2.5-hour period. Figure 2 shows the numbers of adults and larvae collected.

Although larval numbers varied somewhat, an analysis of variance at the 5% level showed no significant differences among the larval numbers collected over the entire period.

With adults, however, Tukey's H.S.D. test at the 5% level showed that the numbers collected at 4:30 and 5:00 A.M. were significantly different from all the rest except those collected at 5:15 A.M. Also, those numbers collected at 4:30, 5:00, and 5:15 A.M. were significantly dif-

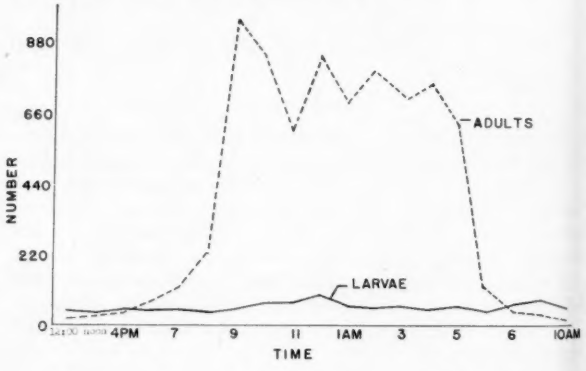

FIG. 1.—Total number of adults and larvae of the alfalfa weevil collected in 120 sweeps taken at periodic intervals over a 24-hour period. Dutchess County, New York, June 30 to July, 1, 1959.

[3] The instrument used was a Bendix-Friez hygrothermograph, model 594.
[4] A Guardian exposure meter (Type PR-2) was used, with a dynacell accessory for dim light. These values are only approximate.

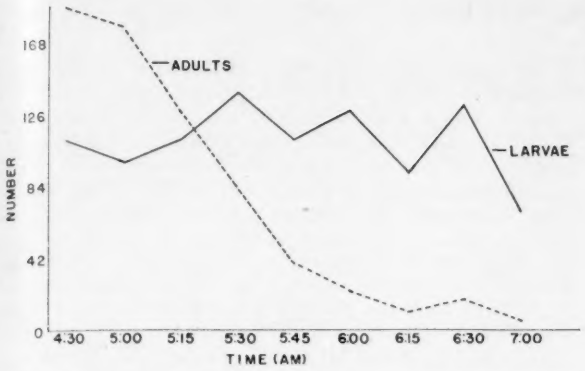

FIG. 2.—Total number of adults and larvae of the alfalfa weevil collected in 300 sweeps taken at periodic intervals over a 2.5-hour period in early morning. Dutchess County, New York, August 1, 1959.

ferent from all except those collected at 5:30 A.M. It may be seen from table 2 that over this 2.5-hour period, temperature and relative humidity varied only slightly, while light intensity increased manyfold.

DISCUSSION.—These results strongly suggest that light intensity is related in some way to the diel behavior of the adult weevil. The majority of adults become active in the evening, crawl up on the alfalfa plants to feed, remain there all night, and then retreat to the base of the plants in the early morning, where they remain most of the day in the debris on the ground, or on the underside of the lower dried-up leaves of the alfalfa plants. The mechanism by which light intensity affects the adults is still not known.

The larvae, on the other hand, do not show changes in activity over the 24-hour period and do not appear to be affected by the normal changes in temperature, relative humidity, and light.

Table 2.—Recordings of temperature, relative humidity, and light intensity in an alfalfa field over a 2.5-hour period in early morning. Dutchess County, New York, August 1, 1959.

TIME (A.M.)	LIGHT INTENSITY (FOOT-CANDLES)	Temperature (° F.)	RELATIVE HUMIDITY (%)
4:30	0.14	58	72
5:00	0.22	57	72
5:15	0.24	57	74
5:30	0.77	56	76
5:45	27.80	56	76
6:00	148.20	56	76
6:15	297.50	56	76
6:30	448.00	56	76
7:00	2,670.00	57	76

From these results, it appears that any surveying for the adult weevils should be carried out in the late evening or during the night in order to get a true estimate of their populations. In order to find greater numbers of individuals, the practice of night-sampling experiments designed for the control of the adult weevils (Koehler *et al.* 1959) is also recommended.

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New Approved Common Names of Insecticides

CARROLL N. SMITH, *Chairman, Committee on Insecticide Terminology*

A revised list of common names of insecticides approved for use in the publications of the Entomological Society of America was published in the April 1959 issue of the JOURNAL OF ECONOMIC ENTOMOLOGY (**52**: 361–2). Changes and additions to the list were published in the October 1959 issue (**52**: 1032). The additional change and the new names listed below have been approved by the Committee on Insecticide Terminology of the Entomological Society of America. The approved common name for the insect repellent *N,N*-diethyl-*m*-toluamide has been changed from "diethyltoluamide" to "deet" because of numerous complaints that "diethyltoluamide" was too long for a common name, that it was not easily remembered, and therefore did not serve the intended purpose. The other new names refer to newly developed fruit fly lures. Trimedlure is an attractant for males of the Mediterranean fruit fly, and the common name is derived from its relationship to medlure, the new compound being a tertiary ester. Cue-lure is an attractant for males of *Dacus cucurbitae* Coq., and the common name was coined from the "Q-sound" of the species name.

Delete:

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS
diethyltoluamide	*N,N*-diethyl-*m*-toluamide	

Add:

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS
cue-lure	acetate of 4-(*p*-hydroxyphenyl)-2-butanone	ENT-31833
deet	*N,N*-diethyl-*m*-toluamide	diethyltoluamide
trimedlure	*tert*-butyl 4 (or 5)-chloro-2-methylcyclohexanecarboxylate	ENT-31560

SCIENTIFIC NOTES

X-Ray Microscope to Study Behavior of Internal-Infesting Grain Insects[1]

JOHN R. PEDERSEN[2] and R. A. BROWN[3]

X-ray inspection has been used for several years in the detection of insect infestations in small seeds and cereal grains. Yuasa (1926), Fenton & Waite (1932), and Shevchenko (1937) showed that this medium could be used effectively in examining seeds of various plants for insect infestations. Milner *et al.* (1950) applied x-ray techniques to detect internal insect infestations in grain on a commercial basis. At present x-ray inspection is widely used by commercial cereal grain handlers for mass detection and estimation of infestations. The purpose of this note is to point out application of another more sensitive x-ray technique for biological studies of individual insects infesting cereal grains internally.

METHODS.—A grain inspection unit[4] was adapted for use in insect development and behavior studies by the Department of Entomology, Kansas State University, during the summer of 1958. Photographic enlargements were made from radiographs to facilitate study. This method has certain restrictions in that sharpness, detail and contrast are impaired in the x-ray and enlargement processes. Radiographs of infested wheat kernels made with the X-Ray Microscope,[5] however, showed greater detail and sharpness. Magnification up to 400× can be obtained. The instrument operates up to 20 kilovolts at 100 microamperes.

The X-Ray Microscope used is an electrostatic beam-focusing instrument. It employs a point source of x rays for shadow projection to magnify and reveal the insect forms inside grain kernels. A narrow cone of electrons emerges from the electron gun and enters a condenser lens and is collimated. The collimated beam enters an objective lens which produces a reduced image of the electron source at the principal focus of the lens. This reduced electron source produces an equally small x-ray source on the target, a thin tungsten-coated beryllium window. The image is then projected onto a viewing screen or film.

Various stages of development of rice weevils, probably the "lesser rice weevil," *Sitophilus sasakii* (Tak.) (Floyd & Newsom 1959) in wheat were selected from laboratory cultures and radiographed with the grain inspection unit and the X-Ray Microscope. Radiographs were made with the grain inspection unit on Type M Kodak Industrial X-ray film exposed for 1½ minutes with 20 kilovolts at 5 milliamperes. Enlargements were made on Kodabromide F-3 paper. Each specimen was subsequently radiographed with the X-Ray Microscope on Polaroid 200 film with an exposure of 20 kilovolts at 40 microamperes for 45 seconds.

RESULTS.—An X-Ray Microscope radiograph of a kernel containing a fourth instar larva is shown in figure 1 (A). Various sections of the kernel embryo as well as hairs at the brush of the kernel may be seen clearly. A radiograph of the same kernel made with the grain inspection unit did not show as much detail, especially at the tips and edges of the kernel, figure 1 (B). On the last day of the fourth instar the larva assumes a distended shape showing the differentiation into the three body regions—head, thorax and abdomen. They are clearly shown in figure 1 (C) on the right side of the kernel. Segmentation is also readily ob-

[1] Contribution No. 758, Department of Entomology, Kansas State University, Kansas Agricultural Experiment Station, Manhattan. Supported in part by research grant E-1942 from National Institute of Allergy and Infectious Diseases, Department of Health, Education and Welfare, Public Health Service. Partial cost of publication of this paper was met by the Chicago Apparatus Company, Chicago, Ill. Accepted for publication May 2, 1960.

[2] Instructor and Assistant Entomologist, Kansas Agricultural Experiment Station, Manhattan.

[3] Assistant Technical Director, Chicago Apparatus Company, Chicago, Ill.

[4] General Electric Grain Inspection Unit.

[5] General Electric X-Ray Microscope. (Radiographs made by R. A. Brown, Chicago Apparatus Company.)

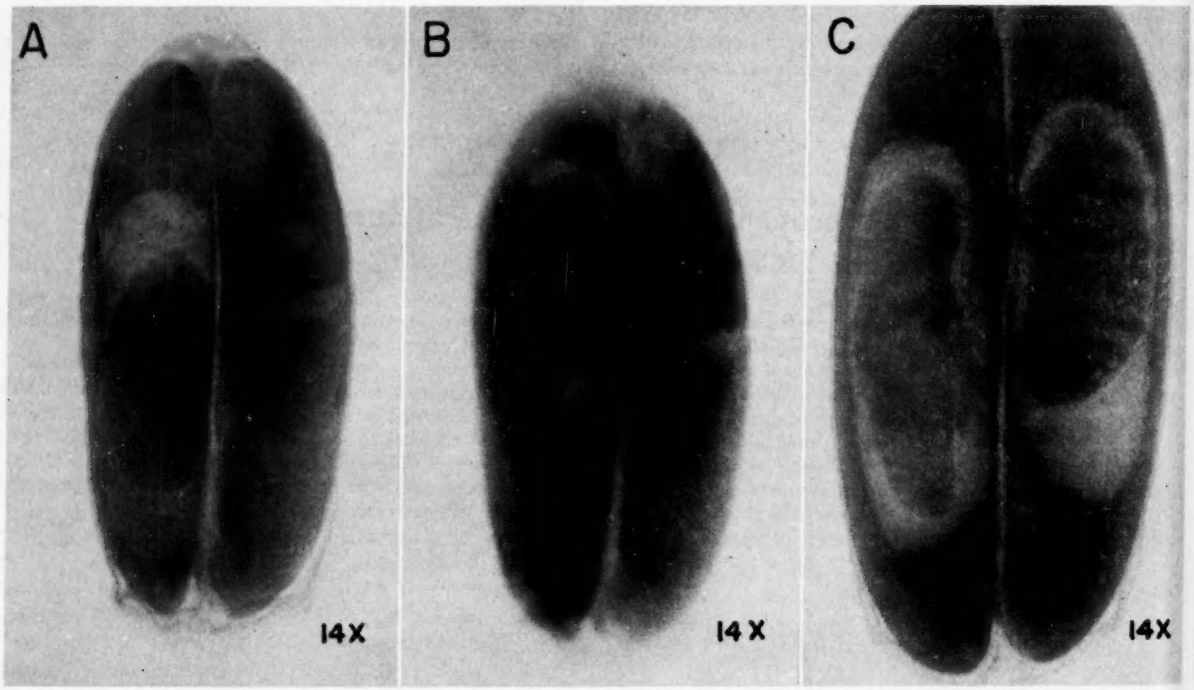

FIG. 1.—X rays of wheat kernels infested with rice weevil. X-Ray Microscope radiographs showing: (A) fourth instar larva, tunnelling filled with frass, position of egg before hatching (upper right) and unused egg cavity (center right) and (C) prepupa (right) and pupa (left). (B) same kernel as (A) radiographed with the grain inspection unit.

served. The left side of the kernel shows the general outline of the pupa.

The X-Ray Microscope has produced pictures far superior to those obtained by previous x-ray techniques used in this laboratory. It makes possible more comprehensive studies of the behavior, development and responses to environmental conditions than were previously possible, by providing sharper and more detailed pictures of greater enlargement.

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The Abundance of Wild Bees (Apoidea) in the Northeastern United States[1]

ROGER A. MORSE, *Cornell University, Ithaca, New York*

During the past 3 years (1957–1959) we have made extensive collections of wild bees in New York, Vermont, New Hampshire, and Massachusetts. The main objective of the project was to determine which wild bees are present in the Northeast and to study their role as pollinators.

In many areas of the Northeast and especially around Ithaca, New York, large acreages formerly devoted to farming are being abandoned and weeds and brush are growing on the land. Many of these abandoned fields have good drainage and provide suitable nesting sites for wild bees. At the same time there are many species of wild flowers, especially dandelion (*Taraxacum* sp.), yellow rocket (*Barbarea* sp.), wild berries (*Rubus* spp.), clovers (*Melilotus* spp. and others) and goldenrod (*Solidago* spp.) which provide abundant forage for oligotropic and polytropic bees.

A 12-inch muslin sweeping net was used for collecting until late 1958 and 1959 when a conventional 15-inch nylon net was used. It has been suggested that a heavy cloth net displaces air in a manner that prevents some of the smaller, lighter weight bees from being caught in the net. In sampling populations the collector walked rapidly, taking wide sweeps at the flower level. When the total volume of collected material (debris and bees) amounted to about a quart, the collector interrupted sweeping to fumigate the material. A cyanide jar made from a standard 1-gallon wide-mouthed jar was ideal for this purpose. When the bees were sorted from the debris it was frequently necessary to place them in a smaller cyanide jar for a short time to complete the fumigation. The large jar was not very efficient when it contained a great deal of fresh, green debris.

During 1958, in approximately one-half of the collections, records were kept on the number of honey bees versus the number of wild bees collected. The resulting figures show that 3395 wild bees were collected while only 1628 honey bees were found. Because of these figures more data concerning this ratio was taken in 1959.

Data from 1959 show that only 716 of 12,073 wild bees collected visited plants that were not visited by honey bees (*Apis mellifera* L.). The number of honey bees as well as the number of wild bees was recorded on flowers visited by both. There was a total of 6,386 honey bees taken while 11,357 wild bees were collected. Most of the collections were made in New York State, especially Tompkins County. The total figures, however, include collections made in Vermont, New Hampshire, and Massachusetts. It would thus appear that in much of the Northeast the number of wild bees is very large and perhaps twice that of honey bees.

The largest wild bees which occur in the Northeast are the bumble bees (about 14 species) and the common carpenter bee, *Xylocopa virginica* L. Megchilidae are not very common, at

[1] Accepted for publication February 24, 1960.

Table 1.—Dates, host plants, locations, and weight of some collections of bees made in 1959.

DATE	LOCATION (Co. in N. Y.)	DISTANCE FROM APIARY[a] (YD.)	WILD BEES No.	WILD BEES Weight (gm.)	HONEY BEES No.	HONEY BEES Weight (gm.)	NECTAR OR POLLEN HOST PLANT
May 7	Tompkins	880	52	0.916	21	2.280	*Melilotus officinalis*
18[b]	Tompkins	200[c]	219	4.335	9	0.945	*Barbarea vulgaris*
June 5	Tompkins	880	40	1.452	83	7.250	*Lotus corniculatus*
8	Tompkins	880	93	1.634	1	—	*Rubus* sp.
9	Tompkins	100	40	1.930	67	5.725	*Lotus corniculatus*
10	Otsego	?	89	2.773	9	0.687	*Melilotus officinalis*
20[b]	Tioga	?	72	3.407	59	5.025	*Melilotus officinalis*
20[b]	Tioga	?	36	1.202	53	4.260	*Melilotus officinalis*
23[b]	Tompkins	1,760	226	2.871	603	50.350	*Melilotus officinalis*
24	Broome	?	18	0.305	41	3.300	*Melilotus alba*
26	Tompkins	1,760	91	1.444	323	25.800	*Melilotus officinalis*
28	Tompkins	880	1,255	5.143	58	4.886	*Melilotus alba*
July 22[d]	Tompkins	200	78	0.382	61	5.280	*Medicago sativa*
23[b]	Tompkins	200	142	0.931	82	7.010	*Medicago sativa*
27	Tompkins	200	176	0.723	193	15.992	*Medicago sativa*
30[d]	Tompkins	880	94	0.585	33	2.785	*Medicago sativa*
Aug. 1	Tompkins	880	328	2.055	61	5.325	*Medicago sativa*

[a] Containing 30 to 45 colonies of honey bees.
[b] One bumble or carpenter bee was taken on May 18, June 20, 23, and July 23. Three were taken on June 20 (second collection). These weights and numbers are not included in the table.
[c] This apiary contained only 10 colonies of honey bees.
[d] Collections made on July 22, 23, and 27 were in the same area as those made on July 30 and August 1.

least on the more common plants, and less than 200 of 12,000 wild bees collected in 1959 belong to that family. Halictidae are by far the most common, probably composing 60% to 70% of the total. Of these *Dialictus* are the most common species. Of 4,328 Halictidae collected in 1957 and 1958 (collections from Massachusetts, New Hampshire and New York) there were 746 *Dialictus inconspicuus* Smith, 424 *Dialictus nymphaearum* Robertson, 397 *Dialictus viridissimus* Smith, and 396 *Dialictus admirandus* Sandhouse. Several other *Dialictus* were present in lesser numbers. *Dialictus* are small bees; where a large number of wild bees weigh very little the collection usually contains a large percentage of this genus (table 1). Andrenidae represent 20% to 25% of the wild bee population. The other groups are of minor consequence except for *Bombus* and *Xylocopa* which are important because of their size.

After sorting the honey bees and wild bees from the debris they were easily handled and when it was convenient they were weighed before they were pinned. The honey bees were discarded after weighing. The weights are thus of fresh bees and vary according to the amount of nectar and pollen being carried. The added weight of nectar and pollen in the case of both honey bees and wild bees should be relative where both are working on the same plant. (It may be necessary to qualify this last statement after further investigations.) The values shown in table 1 are therefore considered meaningful.

The data in table 1 show that in some instances the wild bees not only outnumber the honey bees but the total weight of wild bees exceeds the total weight of the honey bees collected. In some areas therefore the wild bees offer strong competition to honey bees. It would also appear, that in certain cases, honey bees do not offer such strong competition that the number of wild bees is reduced to very low levels. Statements to this effect have appeared in the literature but more work is needed to determine which population, if either, might dominate.

Present information indicates that having a large number of wild bees in an area depends more on the number of host plants and available nesting sites than anything else. Good collections have been made near apiaries containing 10 to 45 colonies of honey bees.

Conclusions reached in this paper are tentative until more collections have been made in more locations in the Northeast. All specimens of wild bees taken are being kept and labeled with pertinent data including host plant information. It is expected that the project will continue for several years. Only a small percentage of the collections made to date have been indentified to species, making further analysis impossible at this time.

The Life History and Seasonal Occurrence of the Peach Twig Borer in Texas[1]

D. R. KING and T. E. DENMAN[2]

The peach twig borer, *Anarsia lineatella* Zell., is the major pest of peaches in central Texas. Although it is present in the eastern portions of the State, it is usually of minor significance. Complaints of erratic control by peach growers in the central area of the State led to an intensification of the research program on this pest. Seasonal life history studies were conducted during 1958 and 1959 to determine the periods during which control sprays could be applied most advantageously. The data obtained were supplemented by fragmentary information acquired by previous observations.

The peach twig borer passes the winter as an immature larva in a hibernaculum beneath the bark. The larvae became active in early February during the years in which this study was performed. After feeding beneath the bark until the trees were in the pink-bud stage, usually early March, the larvae left their hibernacula and moved upward to tunnel in the developing shoots. First evidence of larval activity was the appearance of wilted shoots when early flowering varieties were in full bloom and foliage was beginning to emerge.

Before the larvae completed feeding, corrugated paper bands were placed around small branches below the wilted shoots. Upon attaining maturity, the larvae migrated to the branches and pupated under the bands. Pupation was initiated in early April and continued into early May. The pupal period ranged from 14 to 23 days with a median of 22 days. Prior to emergence of the moths, the corrugated paper bands were removed from the limbs and placed outdoors in quart ice cream cartons with wire screen lids. Most adults appeared during a 2-week period in late April and early May.

Each day the newly emerged moths were divided into groups of five to 10 and transferred to fresh ice cream containers. A cotton-stoppered vial containing a solution of 1 part sugar to 6 parts water was placed in each carton.

Daily records were kept of the number of eggs deposited. As each adult died, it was removed from its container and its sex determined by examination of the frenulum.

In captivity, the moths of the overwintered generation lived from 3 to 36 days. The adult life span averaged 21.5 days. The females lived longer than the males. After a mean preoviposition period of 3 days, the females deposited an average of 90 eggs each. A definite oviposition site preference was exhibited by females in captivity. When they were placed in quart ice cream cartons and provided with pieces of black cloth for egg-laying, they deposited 92% of the eggs on the cloth and the remaining 8% on a waxed-paper lining placed inside the carton. The addition of a twig with leaves and one or two green peaches in the stage of development commensurate with the season (about $\frac{1}{2}$ to $\frac{3}{4}$ inch in diameter) caused a definite change in oviposition habits. Five per cent of the eggs were deposited on the paper linings; 4% were deposited on the black cloth; less than 1% on the leaves and twigs and 91% on the young peaches. Egg deposition reached a peak in the first half of May but continued until early June.

Four to 7 days were required for the first generation eggs to hatch. Immediately after deposition the eggs were white, but they turned orange on the second day and remained this color until 1 or 2 days before hatching. At this time they turned milky white and the heads of the tiny larvae were clearly visible under magnification. Each day during the period of egg deposition, the paper linings, cloth, and twigs bearing leaves and peaches were replaced. The peaches upon which eggs were deposited were cut into sections and wired to trees in the orchard so that the larvae could make their way to twigs, shoots, or fresh fruits. Even though the vast majority of eggs were deposited on the fruits, the first generation larvae almost invariably moved to the shoots to feed.

Jones (1935) and Bailey (1948) in detailed life history studies in California observed that some larvae of this and succeeding summer generations feed in cells constructed beneath the bark before moving to the shoots to complete development. This habit increased the time required for the larvae to reach maturity. In central Texas, few of these bark-feeding larvae were observed. As a result, their behavior was not studied.

In rare instances, larvae fed briefly on the green fruits before moving to the shoots. Apparently the fruit is not attractive until after the pit-hardening stage. When the fruits of early ripening varieties such as Dixired and Cardinal matured in late May or early June, which is earlier than usual, they were attacked by the larvae. Approximately 3 weeks were required for the completion of the larval stage. Pupae were collected in corrugated paper bands on the trees from late May to mid-June. Four to 10 days with a median of eight were passed in the pupal stage. The first generation adults, which emerged during the first half of June,

[1] Technical Contribution No. 3444, Texas Agricultural Experiment Station, College Station. Accepted for publication February 26, 1960.

[2] Respectively, Associate Professor, Department of Entomology and Associate Horticulturist, Substation No. 20, Stephenville, Texas.

lived and continued depositing eggs until mid-July. The females laid an average of 144 eggs, the majority of which were deposited by late June. Longevity of the moths ranged from 8 to 27 days with an average of 17.4 days.

When females of the first generation were placed in cartons containing black cloth and a twig with leaves and fruit, they exhibited a preference for the fruit similar to that illustrated by adults of the overwintered generation. Seventy-three per cent of the eggs were deposited on the peaches, 22% on the paper lining the carton, 3% on the leaves and 2% on the twigs. The eggs hatched in 5 to 6 days and the second generation larvae became active in mid-June and early July. Peach varieties which ripen in the Burbank July Elberta season frequently suffer heavy damage from this generation of larvae. On trees of other varieties on which the fruit had been harvested or was not yet ripe, the larvae tunneled in the shoots. Pupae of the second generation appeared during late June and July. Pupation required 4 to 11 days with an average of 7. The majority of second generation moths emerged during the last 3 weeks in July and the peak of egg deposition occurred during the last week in July. An average of 4.2 days was required for the third generation eggs to hatch. In the cartons, 92% of the eggs were deposited on fruit, 5% on the twigs, 1% on the leaves, and 2% on the paper lining.

Owing to the overlapping of second and third generation larval activity in late July and early August, the fruit of the Elberta types which normally ripen during that season was infested with larvae of both generations. By early August, the larvae were feeding on the fruit of late-maturing varieties and on the tender shoots of early-maturing varieties which had recovered from the fruit load and were producing succulent growth. Pupae of the third generation appeared during August and early September with the majority appearing in late August. Third generation adult emergence and egg deposition continued from mid-August until mid-September. In captivity, the moths lived an average of 15.3 days.

During the first 2 weeks of the oviposition period, eggs were deposited on late-maturing peaches. The larvae which hatched from these eggs reached maturity, pupated, and emerged as adults of the fourth generation in mid-September. It is probable that eggs deposited by the adults in late September and October produce larvae of the fifth generation which migrate down the limbs and seek hibernation quarters. Unfortunately, circumstances prevented the continuance of these studies after the fourth generation pupae transformed to adults.

Eggs deposited by the third generation moths during the latter half of the oviposition period in early September were placed on leaves and twigs since fruits were no longer available. Larvae which hatched from these eggs fed for a brief period on the terminals and then moved to hibernacula under the bark where they fed until winter. Beginning in mid-September, hibernacula were found in crotches of trees.

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On the Flight Distance of the Boll Weevil[1]

C. M. BECKHAM and L. W. MORGAN[2]

There has been considerable need for conducting detailed population studies on the boll weevil, *Anthonomus grandis* Boh., under isolated conditions. In addition, there has been some discussion of maintaining selected strains of the boll weevil under field conditions in locations free of contamination from other strains. The latter has been of interest in relation to studies on resistance of this insect to certain insecticides. The question frequently arises as to how far the boll weevil can fly or how far such test areas would need to be from infested cotton to prevent contamination.

A review of the literature shows several references concerning the distances of flight or movement of the boll weevil. Hunter (1923) stated, "Since 1894 the boll weevil has extended its range annually from 40 to 160 miles. The boll weevil moves from place to place by flight. Although a weak flyer compared with many insects, it has been known to cover a distance of more than 40 miles in a very short time. Its flight cannot be prolonged, but successive short flights, especially in connection with favorable winds, often carry the insect considerable distances." Harned (1910) discussed the eastward spread in Mississippi during 1909 of the weevil from the western part of Pike County to the eastern part of Green and Jackson Counties, or approximately to the Alabama line—about 120 miles. Worsham (1916) stated that since the boll weevil appeared at Brownsville, Texas, in 1892, it had advanced steadily northward and eastward at the rate of about 65 miles per year. Hunter & Hinds (1905) stated, "It appears that exactly these conditions of defoliation (by the cotton leaf worm), accompanied by favorable weather conditions, prevailed in east Texas during the autumn of 1904, and it is possible that they account in some degree for the long flights, which must have been taken by multitudes of weevils, covering in the course of this migratory movement a strip 30 to 70 miles in width." The same authors further discussed the breadth of a newly infested strip in early October, from the Texas line into Southern Louisiana, that averaged about 50 miles, and in some portions reached between 60 and 70 miles. These references indicate a progressive advancement of the boll weevil after individual movements of the several generations as they developed during the season. However, Hunter & Hinds (1905) gave a rather specific distance of flight when they further stated, "One of the most striking facts observed was the weevils succeeded in crossing bodies of water which, in some cases, were fully 10 miles in breadth. Stretches of non-cotton-production country were also passed which were fully 40 miles in breadth."

Entomologists of the Division of Entomology, University of Georgia College of Agriculture Experiment Stations, have been interested in finding a site in Georgia that would be isolated from the boll weevil in order to conduct studies on population dynamics, habits, hibernation, flight distance, et cetera, on this insect. Because of the reduction in cotton acreage during recent years, it was considered likely that an isolated location could be found. A site on the northern end of St. Simons Island was selected where cotton could be grown and a preliminary study was made during the 1959 season to determine whether the location was isolated. Historically, this island was a major cotton producing area many years ago, however, no commercial cotton has been planted recently. The last known date cotton was planted prior to 1959 was in 1954; it was planted for ornamental purposes as a tourist attraction at a resort hotel on nearby Sea Island.

PROCEDURE.—St. Simons Island is located in the southeastern part of Georgia along the Atlantic Coast. The site where cotton was planted for this study is approximately 15 miles from the city of Brunswick in Glynn County. The island is separated from the mainland by several rivers and marshland.

Approximately 1/10 acre of cotton was planted at the site on May 13. The planting consisted of three rows 125 feet long for each of the following varieties: Coker 100 W, Empire, Plains, and Coastland RN, the latter a Sea Island variety. The recommended practices of fertilization and cultivation were followed as

[1] Paper No. 374, Journal Series, Georgia Experiment Station, Experiment. Accepted for publication February 26, 1960.

[2] Entomologist, Georgia Experiment Station, Experiment, and Assistant Entomologist, Georgia Coastal Plain Experiment Station, Tifton, respectively.

Appreciation is expressed to Mr. R. A. Taylor on whose farm this study was made.

nearly as possible. In addition to the initial fertilization and subsequent application of nitrogen, a second application of a complete fertilizer was made during the season to insure continuing good growth. A good stand was obtained. The plants grew well and by the first of July were squaring and blooming freely.

The cotton was examined at about weekly intervals until July 24 for presence of boll weevils and signs of their activity. No signs of the boll weevil were found up to this date. The cotton was not examined again until August 26 at which time all varieties were still squaring; however, a few bolls were also opening. The Sea Island variety especially was squaring freely. On this date, a total of 28 squares with egg or feeding punctures resembling that caused by the boll weevil, were collected and caged for rearing of adults. Two of the squares contained nearly mature larvae. No adult boll weevils were observed in the cotton on this date. A total of 10 adults emerged from the infested squares during early September.

Subsequent examinations of the cotton planting on September 2, 11, and 23 did not reveal any adult weevils in the field. However, a very few punctured squares and signs of feeding on blooms were observed during this time. The first adults were found in the field on October 8. These weevils apparently had developed in the cotton in this field. A few punctured squares collected on October 8 subsequently produced adults. A square-infestation count in the field on November 5 showed 22% infestation.

DISCUSSION.—Since infested squares were found on August 26 and two contained nearly mature larvae, it is considered that the boll weevil migrated to this cotton about August 15. The U. S. Department of Agriculture Agricultural Stabilization and Conservation Offices in the counties near Glynn County were consulted to determine the closest planted cotton allotments to the test site. There were no cotton allotments in Glynn County and no cotton was officially planted. Although there were a few small acreage allotments in McIntosh and Camden (counties to the north and south of Glynn, respectively), no cotton was planted in these counties during 1958 or 1959. The cotton nearest to the test site planted during 1959 was in Wayne and Brantley Counties. These counties border Glynn on the northwest and west, respectively. The airline distance from the closest field in Wayne County from the site on St. Simons Island was approximately 34 miles in a northwesterly direction, while the distance to the Brantley County field was about 25.5 miles in a westerly direction. The weevil infestation in the cotton on St. Simons Island apparently originated from one of these fields. The fields in Wayne and Brantley Counties were examined on August 27 and both were moderately infested with the boll weevil.

This study shows that for a planting of cotton to be isolated from the boll weevil, it must be removed from other cotton by a distance greater than 25.5 miles. Results indicate that under similar conditions in this area of Georgia, population studies on the boll weevil may be conducted until about August 15 without contamination by migratory weevils.

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Parasitism of the Overwintering Pupae of the Melon Leaf Miner, *Liriomyza pictella*[1]

EARL R. OATMAN[2]

Field and laboratory investigations indicated that the melon leaf miner, *Liriomyza pictella* (Thomson), overwintered as puparia in the Patterson area of the San Joaquin Valley of California (Oatman & Michelbacher 1958, 1959).

Large samples of Cranshaw and honeydew melon leaves infested with high population of melon leaf miner larvae were collected November 1, 1955, from remnants of plants just before the melon fields were plowed in preparation for winter crops. The leaves were placed in field crates constructed of wood slats spaced ½ inch apart to provide adequate ventilation and exposure to the natural environment. Lawn cloth was used to line the bottom and extended part way up the sides and ends to retain the emerging larvae. The open tops of the crates were covered with lawn cloth and firmly secured. Within 1 week, most of the larvae had emerged from the leaves and formed their puparia. The crates were left outside near the fields until March 3, 1956, at which time they were transferred to the insectary. Here, the contents were removed, placed in rearing cages, and held for 2 weeks at room temperature for adult emergence.

No leaf miner adults emerged from the hundreds of puparia present. Many of the puparia were shriveled and blackened as if from winter mortality. A total of 366 specimens of hymenopterous parasites was collected. These were sorted and sent to the Insect Identification and Parasite Introduction Section of the U. S. Department of Agriculture, Beltsville, Maryland, where they were determined by B. D. Burks and C. F. W. Muesebeck.

Six species were represented, 3 in the family Eulophidae; 2 in Braconidae; and 1 in Pteromalidae. All but one species had been previously reared from the melon leaf miner in 1954 and 1955 (Oatman 1959). The species in order of abundance were as follows: *Halticoptera aenea* (Walk.); *Chrysocharis ainsliei* Cwfd.; *Opius suturalis* Gahan; *Opius* sp. (apparently a new species); *Derostenus arizonensis* Cwfd.; and *Chrysocharis parksi* Cwfd.

H. aenea accounted for 66% of the total specimens. The two species of *Opius* and *Chrysocharis* represented 16% and 15% of the total, respectively. Records obtained in the parasite studies of *L. pictella* in August and September of 1954 revealed that *H. aenea* accounted for 64%, 80% and 87% of the total number of parasites reared from three collections of heavily mined Cranshaw melon leaves from two fields. *H. aenea* is apparently the most important parasite in the natural suppression of the melon leaf miner, especially in the latter part of the growing season when parasitism approaches 100% (Oatman 1959).

Although adult leaf miners were not obtained, the presence of the parasites in the puparia from November 1955 to March 1956 is evidence that *L. pictella* overwinters as puparia in the soil and plant debris.

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[1] Accepted for publication March 7, 1960.

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Systemic Insecticides as Feed Additives for Cattle Grub Control[1]

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A number of workers have demonstrated that single oral administrations of ronnel and DOWCO 109 will give satisfactory

control of cattle grubs (*Hypoderma lineatum* (De Vill.) and *bovis* (L.)). Extensive tests have also been conducted with these two insecticides as feed additives, to determine whether small daily dosages would be as effective as a single administration. Studies of this kind have been conducted by Knapp *et al.* (1958), DeFoliart *et al.* (1958), Howell *et al.* (1958), French *et al.* (1958), and Drummond & Graham (1959), but their results were quite variable.

In 1957 and 1958 tests were conducted at Kerrville, Texas, with repeated small dosages of various systemic insecticides in the feed of cattle. Ronnel (Trolene) was tested in 1957, and dimethoate, Co-Ral® (*O*-(3-chloro-4-methylumbelliferone)*O,O*-diethyl phosphorothioate), DOWCO 105® (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl ethylphosphoramidothioate), Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and DOWCO 109® (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) in 1958. The results are presented herein.

TESTS IN 1957.—Two yearling Hereford steers were fed 25% ronnel formulated in a wettable powder daily starting July 24. One was fed 1 mg. of the active ingredient per kg. of body weight for 42 days, and the other 3 mg./kg. for 70 days. The treated cattle and six untreated animals were examined monthly from August through January. As grubs encysted, their locations were marked on an outline map of the back of each animal, and cumulative counts were thus obtained. The animal treated at 3 mg. had only one grub, but 24 were recorded for the one treated at 1 mg. The untreated cattle were infested with an average of 33.5 grubs each.

TESTS IN 1958.—Two groups of Hereford cattle were treated. One group consisted of 2-year-old heifers and steers from South Dakota, which had been on pasture at Camp Stanley, Texas, during the heel fly season. Eight of these animals were fed 10% dimethoate from a feed concentrate at two dosages and 16 were fed Co-Ral as technical material at four dosages for various periods. The animals were treated in the latter part of July and first part of August, about 6 weeks before grubs started to appear in their backs. All animals consumed treated feed readily.

The second group were short yearling steers and heifers, purchased in Wyoming and moved to Kerrville, Texas, in October. Twenty cattle were placed in groups of four and offered feed treated with the following insecticides beginning on December 1: 25% DOWCO 105 from a wettable powder, technical Co-Ral, 50% Dipterex from a soluble powder, 10% dimethoate from a feed concentrate, and 25% DOWCO 109 from a wettable powder. Normal ration was 3 to 6 pounds of ground feed containing crushed shelled corn, crimped oats, coarse ground alfalfa hay, cottonseed and bone meal, and salt.

All cattle in the second group were fed the ration only 2 days before the insecticides were added, but some apparently did not become adjusted to the feed-lot diet. The animals offered DOWCO 105, Co-Ral, and DOWCO 109 ate the feed readily. Three animals ate feed containing dimethoate, but one did not eat for 2 days and feeding time was extended for 7 instead of 5 days. One animal ate Dipterex in the specified time, two refused feed for 2 days, and one required 17 days to consume the dose.

Cooperating veterinarians[2] observed no ill effects in either group due to treatment, but noticed some depression in blood cholinesterase values. Cattle in both groups were examined monthly during the period of grub emergence. Grubs appeared in the first group about 2 months after treatment and in the second in less than 1 month. Results of tests are summarized in table 1.

In the first group both dimethoate treatments were effective. Co-Ral was considerably less effective, the degree of control appearing to be neither a function of length of treatment or dosage.

All treatments in the second group were highly effective. The animal that was slow to consume dimethoate had two grubs; three others that ate normally had a total of only one grub. All animals treated with Co-Ral were infested; 10 of the grubs were from one animal. This treatment was much more effective than those with Co-Ral in the first group. More total insecticide was administered, and also treatments were made closer to the time when grubs would appear in the animals' backs. The animal that took 17 days to consume the feed treated with Dipterex had one grub; the others were grub-free. The DOWCO 105 and DOWCO 109 treatments afforded 100% control.

Table 1.—Effectiveness of several systemic insecticides against cattle grubs when used as feed additives, 1958.

Insecticide	Dosage (Mg./Kg.)	Days Treated	Number of Cattle		Average No. of Grubs per Animal	Control (%)
			In Test	Infested		
			Group 1			
Dimethoate	15	1	4	4	3.5	81
	7.5	2	4	3	2.2	91
Co-Ral	20	1	4	2	11.5	50
	10	2	4	4	24.0	0
	4	5	4	4	17.8	26
	4	10	4	4	13.2	40
Untreated			26	26	23.9	—
			Group 2			
Dimethoate	5	5	4	2	0.7	99
Co-Ral	10	5	4	4	3.5	91
DOWCO 105	15	10	4	0	0	100
DOWCO 109	20	5	4	0	0	100
Dipterex	10	10	4	1	0.2	99
Untreated			21	21	38.8	—

In these tests emphasis was placed on feeding for short periods, so that administration would be less complicated and residues would be minimized. Short feeding periods may be of value to feed-lot operators when starting new groups of cattle at certain times of the year, and to ranchers, who often have their cattle gathered for 2 to 3 days a few times each year. Grub control can be obtained by feeding certain compounds for short periods, but more research to determine timing, periods, safe dosages, residues, and other facets of the feeding operation must be conducted before practical application can be made.

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[1] Accepted for publication March 11, 1960.

[2] R. D. Radeleff and J. B. Jackson, Animal Disease and Parasite Research Division, Agric. Res. Serv., U.S.D.A.

Mined Buds, a Hibernation Site for Spruce Budworm[1]

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Preference by first-instar larvae of the spruce budworm (*Choristoneura fumiferana* (Clem.)) for empty staminate flower bracts of balsam fir as overwintering sites has been shown by Bess (1946) and Blais (1952). On the other hand, Jaynes & Speers (1949) and Miller (1958) found no definite evidence of a preferred hibernation site. However, it is generally accepted that reproductively mature trees present conditions that are more favorable to survival of overwintering larvae than do trees in nonreproductive condition, and that nonflowering, immature stands of balsam are less vulnerable to the budworm. Studies by Jaynes (1951) in the Northeast indicated very low overwintering survival on small balsam trees (6 to 10 feet tall). Similar results were obtained by Bean in Minnesota.[3]

Some interesting evidence on this point was obtained in 1959 on the La Croix District, Superior National Forest, Minnesota. Here several areas with advanced growth balsam only (trees 1 to 3 inches diameter breast height) were found that had been heavily infested for 3 successive years. These areas were logged 5 to 15 years ago. Only occasional large aspen, and black spruce less than 5 inches diameter breast height, remain in the overstory. Heavily attacked mature stands are a half mile or more away. Examination of one of these areas in late August showed no trees ever having borne staminate flowers. The scattered small black spruce trees were only lightly defoliated. Closer examination of the small balsam revealed numerous pupal cases and hatched egg masses. Less obvious, but numerous, were vegetative buds hollowed out by heavy budworm feeding during the early part of the growing season. These buds bear a striking resemblance to the empty cup-like staminate flower bracts found on mature balsam (fig. 1).

During September three balsam trees, 11, 13, and 16 feet tall, were cut into convenient pieces and bagged. Examination with a binocular microscope found 15% to 24% of the mined-out buds occupied by hibernating larvae. No budworms were found on the bark, but one was found under a bark scale. About 0.5% of the mined buds harbored more than a single larva.

The number of these overwintering niches per tree varies with the intensity of previous early season defoliation but, on the basis of the small sample taken, upwards of 2,000 of these sites can be found on a tree 11 to 16 feet tall. The number of such sites increases each year since the hollowed buds are persistent.

DISCUSSION.—The use of mined buds for hibernation sites may account for the persistence of budworm infestations in young balsam stands, where large populations do not ordinarily survive from year to year. The first mined buds are caused by heavy feeding of small larvae which probably infest the small trees during spring dispersal. Subsequently, moth flights or drift of first-instar larvae might add to the population already present since favorable overwintering sites are available. How this action by the budworm may affect management of balsam fir will be the subject of further investigation.

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[1] Accepted for publication March 11, 1960.

[2] Maintained at St. Paul 1, Minnesota, in cooperation with the University of Minnesota. Mr. Batzer is stationed at the Grand Rapids, Minnesota, Research Center.

[3] Unpublished data in files at the Lake States Forest Experiment Station, St. Paul 1, Minnesota.

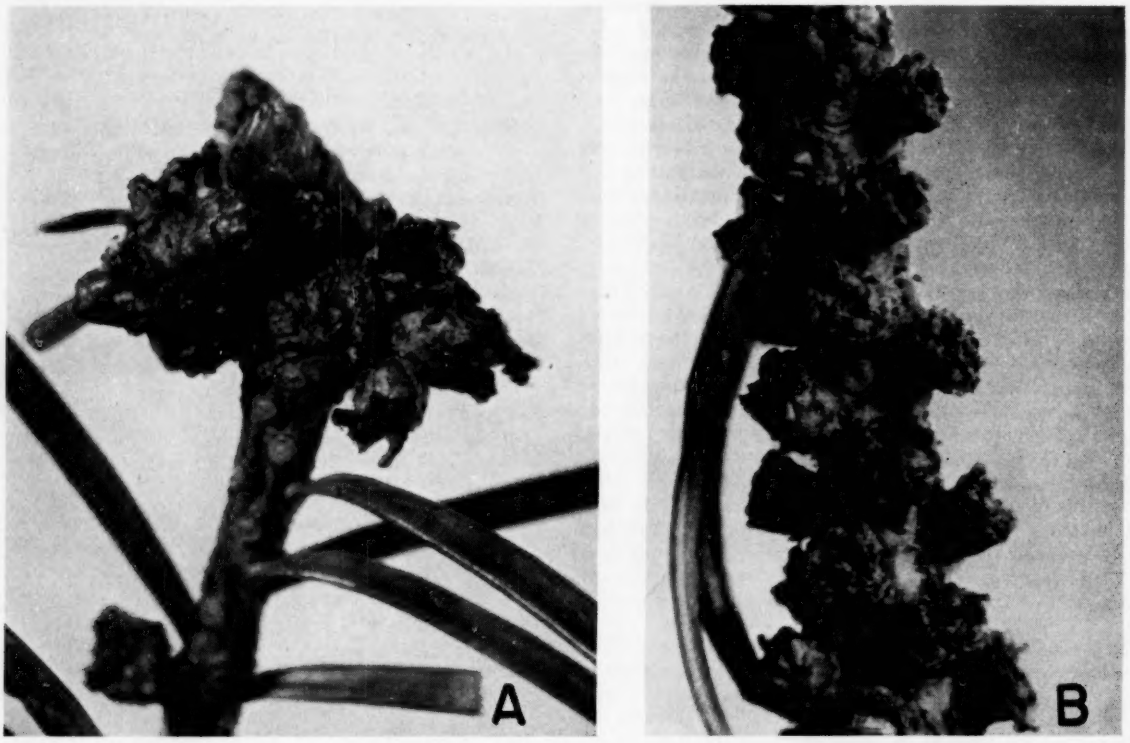

FIG. 1.—Balsam fir twigs showing similarity between (A) vegetative bud hollowed out by spruce budworm larval feeding and (B) empty staminate flower bracts.

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Effect of Urea on the Control of Some Apple Insects with Certain Insecticides[1]

STANLEY C. HOYT[2]

In Washington, urea sprays are sometimes applied directly to apple foliage as a source of nitrogen. Since this material has been included in the early cover sprays along with materials for the control of the codling moth, *Carpocapsa pomonella* (L.), laboratory studies were begun on the compatibility of DDT and urea in November, 1958.

A laboratory colony of codling moth was reared by the method described by Dickson *et al.* (1952) to provide eggs in a stage suitable for laboratory tests. The tests were conducted according to the method described by Barnes (1958) using small metal cells to isolate the individual eggs and force the newly hatched larvae to enter the fruit through uniform insecticide deposits.

Individual Winesap apples were placed on a turntable rotating at 15 r.p.m. and sprayed with an air-pressure laboratory sprayer through one rotation of the turntable. Four cages were then attached to each apple and one codling moth egg was placed in each cage. Twelve apples were used for each treatment in each replicate. Eight replicates were given the following treatments: one pound of 50% DDT wettable powder per 100 gallons of water, 1 pound of DDT plus 5 pounds of urea (45% total nitrogen), and an unsprayed check. Ten replicates were then run using 2 pounds of 50% DDT per 100 gallons of water, 2 pounds of DDT with 5 pounds of urea, and an unsprayed check. The percentage of the codling moth larvae which entered the fruit was then observed for each treatment. This percentage was then adjusted by Abbott's formula to give the per cent reduction of fruit entries over an unsprayed check (see table 1).

The differences between the percentage of reduction in fruit entries obtained with DDT alone and with DDT plus urea at both the 1- and 2-pound rates of DDT were significant at the 1% level. The per cent reduction in entries obtained with 1 pound of DDT was higher than that obtained with 2 pounds of DDT plus urea. It is evident from these results that the urea or some impurity contained in the urea impaired the control of codling moth in the laboratory.

Table 1.—The reduction of codling moth fruit entries with DDT and with DDT plus urea in laboratory tests. Wenatchee, Washington, 1958–1959.

Replicate	Per Cent Reduction of Fruit Entries			
	1 lb. DDT	1 lb. DDT +5 lb. Urea	2 lb. DDT	2 lb. DDT +5 lb. Urea
1	50	18	56	16
2	23	22	83	36
3	29	1	70	28
4	22	0	57	9
5	42	25	65	21
6	25	14	75	49
7	36	18	79	17
8	30	8	59	11
9			49	11
10			86	17
Average	32.1	13.3	67.9	21.5

Table 2.—Residues of DDT when sprayed alone and when combined with urea in laboratory tests. Wenatchee, Washington, 1959.

Date Sprayed (Feb.)	Dosage per 100 Gal. of Water	DDT Residue (P.P.M.)[a]
18	DDT, 2 lb.	1.2
18	DDT, 2 lb.+urea, 5 lb.	1.4
20	DDT, 2 lb.	1.5
20	DDT, 2 lb.+urea, 5 lb.	1.7

[a] Analyzed on February 26, 1959, by the U. S. Public Health Service Communicable Disease Center, Wenatchee, Washington.

Residue analyses of two paired apple samples from these tests showed little difference in the DDT deposits (table 2). This indicates that the effect of the urea is not DDT degradation, since the method of analysis was specific for DDT.

During 1959, field tests were conducted to determine if this difference in control occurred in the orchard. Single tree plots replicated five times were set up in a block of Winesap trees. The trees were sprayed with conventional high pressure equipment and orchard guns at 600 p.s.i. using 20 gallons per tree. Four applications of each treatment were made during the season. One hundred and fifty apples per tree were examined for the presence of worms or stings.

With 2 pounds of 50% DDT wettable powder per 100 gallons of water, an average of 3.7% of the fruit was wormy and an additional 2.9% had stings. Where 5 pounds of urea was added to 2 pounds of DDT in the first two applications an average of 6.9% of the fruit was wormy and an additional 4.5% had stings. Although DDT alone provided better control, the difference was not significant at the 5% level.

Similar field tests were conducted using Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol), and Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone), against the European red mite, *Panonychus ulmi* (Koch), and malathion against the apple aphid, *Aphis pomi* DeG. Urea combined with these materials did not reduce their effectiveness against these pests.

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[1] Scientific paper No. 1944, Washington Agricultural Experiment Stations, Pullman, Washington. Projects 1187 and 1477. These investigations were partially supported by a research grant from E. I. du Pont de Nemours and Company. Accepted for publication March 28, 1960.

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An Inexpensive and Effective Indoor Insectary[1]

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An insectary, designed for use under ordinary room conditions, presently composed of a battery of five large walk-in units each

[1] Accepted for publication March 28, 1960.

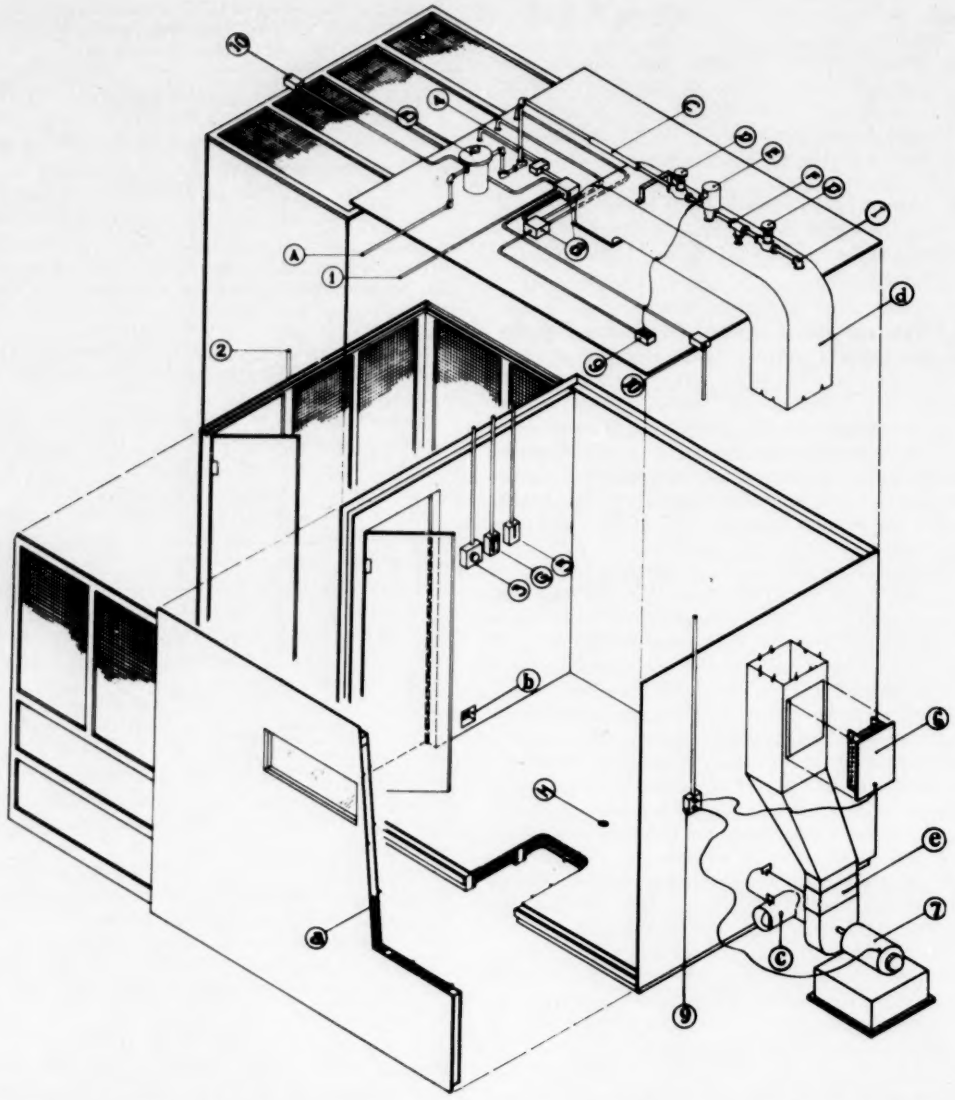


FIG. 1.—Isometric projection of controlled rearing chamber and entrance trap. A. Water intake. B. Filter. C. High pressure rubber hose. D. Hand valves. E. Magnetic valve. F. Fog nozzle in duct. G. Humidistat. H. Floor drain. I. Water line drain. 1. Electric power conduit. 2. Power to outside switch. 3. Variable transformer. 4. Relay, 5 to 6. 5. Thermostat. 6. Electric heater. 7. Blower. 8. Junction boxes. 9. Receptacles. 10. Condulets. a. Plywood panels with glass wool insulation. b. Air vent. c. Return air tee in suction and adjustable dampers. d. Aluminum duct.

with independently controlled temperature, humidity, light, and air circulation, has been used successfully for mosquito (*Culex tarsalis* Coq.) rearing at the Rocky Mountain Laboratory for about 2 years. The essentials of constructing and operating one such unit are described and illustrated in this paper.

Breeding Chamber and Entrance Trap.—Outside dimensions of the controlled chamber (fig. 1) in which caged mosquitoes are maintained are 8 feet long, 6 feet 4 inches wide, and 7 feet 3 inches high. The size was dictated by the largest panels that freight elevator and doorways could accommodate since all units were prefabricated in our carpenter shop. Panels are lap-jointed for tight fit and for easy assembly and dismantling.

Walls and ceiling consist of inner and outer panels of $\frac{1}{4}$-inch plywood separated and supported by $2'' \times 2''$ studding and interlined with 2-inch glass fiber insulation (a).[2] Expanded polystyrene which is inert and fungus-resistant may also be used for insulation. The inner floor panel of $\frac{3}{4}$-inch plywood is supported by $2'' \times 4''$ joists and insulation is backed with Masonite. The floor is designed to slope to a center drain (H). The door, of course, opens inward.

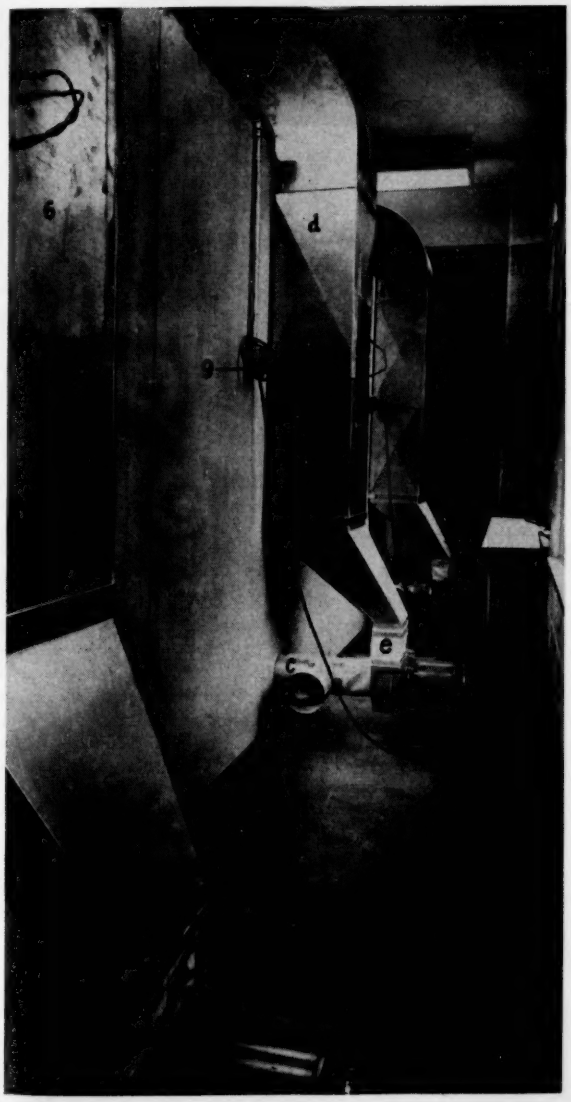
To make the chamber water-proof, the whole interior is lined with glass cloth applied with plastic resin and coated with a white plastic paint.

A screened entrance trap 4 feet deep (figs. 1, 2), where larvae are reared to pupae, is attached to the chamber.

Light.—Illumination within the chamber is provided by a vapor-proof incandescent 300-watt ceiling lamp operated by an outside switch (2). A small window is convenient. Lighting is

[2] Parenthetic numbers and letters apply equally to figure 1 and to corresponding parts visible in figures 2 to 4.

FIG. 2 (*above*).—Entrance traps of two separate breeding units. Labelled parts correspond to figure 1.



controlled manually or automatically, as required, with a variable transformer (3).

Temperature.—Heat is furnished by a 1500-watt unit (6) installed in a 12″×14″ aluminum duct (d) leading into ceiling of chamber from a 1120 r.p.m., 650 c.f.m. blower (7). It is equipped with a return air tee (c) in suction, with adjustable dampers, for maintaining a balance between fresh (note screened air vent, b) and recirculated air. A canvas sleeve (e) breaks the solid connection of duct and blower to reduce vibration inside. Temperature is thermostatically (5) controlled through relay (4) to heater (6).

Humidity.—Water is piped from (A) through filter[3] (B) to high pressure rubber hose (C) to hand valve (D) to magnetic valve[4] (E) to fog nozzle[5] (F) installed in duct—brass with stainless steel pin and S-100 strainer, 0.14 gal. per hr. at 10 lb. to 0.42 gal. per hr. at 100 lb.—to blow-off valve (D) to water line drain (I). The high pressure hose is installed to facilitate lifting

[3] Fulflo Filter, Commercial Filter Corporation, Melrose, Massachusetts.
[4] Minneapolis-Honeywell.
[5] Bete Fog Nozzle Incorporated, Greenfield, Massachusetts.

FIG. 3.—Rear view of three independent units sharing common partitions, showing blower, duct and heater installations. Labelled parts correspond to figure 1.

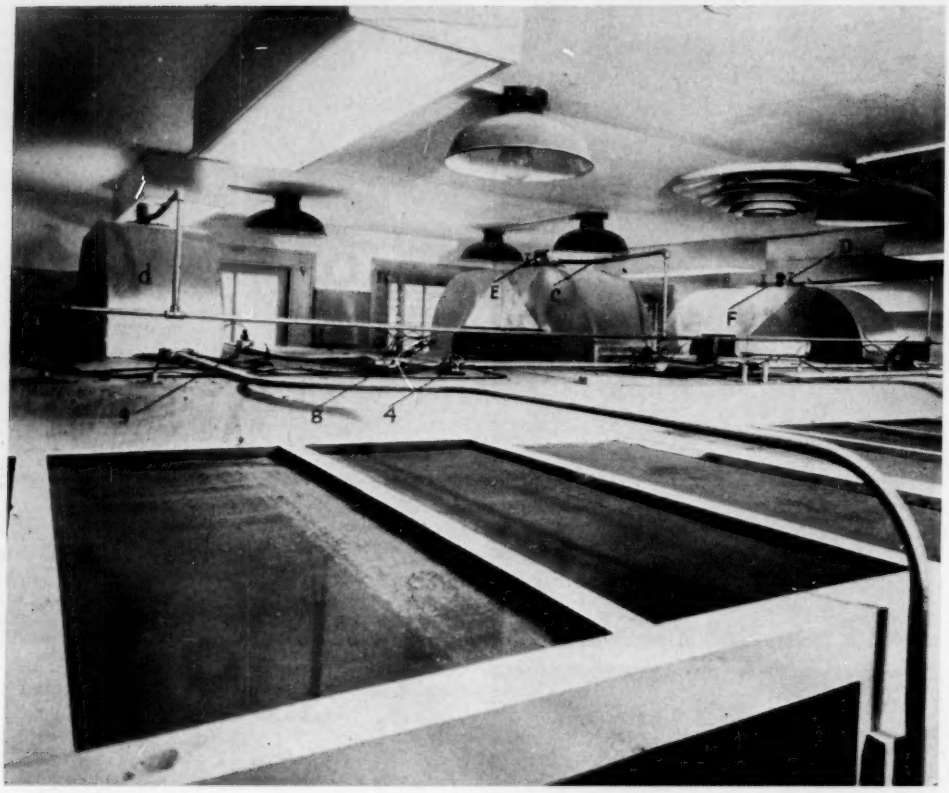


FIG. 4.—Top view of 3-unit section overlooking entrance traps. Labelled parts correspond to figure 1.

assembly for cleaning or replacing fog nozzle. Humidity is controlled by humidistat (G) to magnetic valve (E).

COMMENTS.—In general, the operation of the units described above has been very satisfactory for nearly 2 years. However, as with most controlled units, the humidifying system has occasionally malfunctioned as a result of micro-particles clogging the jet which has an aperture of about 1/1000 inch. We have practically eliminated this difficulty by cleaning and/or replacing jets routinely, in our case, at monthly intervals.

Skill and experience are required for applying glass cloth with plastic resin. The amateur craftsman will save both time and expense by consulting makers of fiber-glass boats, et cetera.

The cost of a complete and smoothly functioning unit need not exceed $1000.00, and with a little care and ingenuity could be somewhat under that figure.

The area occupied by all units is secured by sealing and screening, and by locked doors.

An Abnormal Antennal Condition in a Resistant Strain of the Boll Weevil[1]

JOHN G. THOMAS and J. R. BRAZZEL[2]

On July 18, 1959, a male boll weevil (*Anthonomus grandis* Boh.) of the College Station, resistant (CS, R) strain (Brazzel & Lindquist 1960) was observed in which the clavola of the left antenna was perfectly paired and the right antennal club was cleft. The joints of the funicle beyond the first segment were perfectly duplicated on the left antenna. The club of the right antenna was emarginate, appearing to have only begun duplication, figure 1.

The above individual was found shortly after pupation among a group of weevils reared in the laboratory on a larval diet. It appeared that this character could possibly be used as a genetic marker for resistance. Observations on the forked-antenna male indicated the individual to be normal in all respects—mating freely, feeding and moving about normally.

In hopes of duplicating the forked-antenna condition in the offspring, the male was mated with 12 resistant females to produce an F_1 generation. An F_2 and F_3 generation were also reared and observed. Two backcrosses were made, these included first backcross matings of randomly selected females from the F_1 generation with forked-antenna male, and second backcross matings of randomly selected females of the first backcross progeny with the forked-antenna male. Progeny of the first backcross generation were mated *inter se*.

The progeny of all the above matings were reared on a larval diet according to the procedure described by Brazzel, *et al.* (1959). Observations were made on the following number of progeny; 777 F_1, 543 F_2, 190 F_3, 244 first backcross, 231 second backcross, and 130 *inter se* mating of first backcross individuals.

A total of 2,115 weevils were observed with no evidence of the forked-antennal character being present in any of the offspring. These results indicated that this character was a somatic mutation, was not genetically determined or was the result of an extremely complex genetic mechanism. There is a possibility of a very complex inheritance which is apparently very rare in the natural population causing this condition. Frequently, this type character is associated with a simple type inheritance with only

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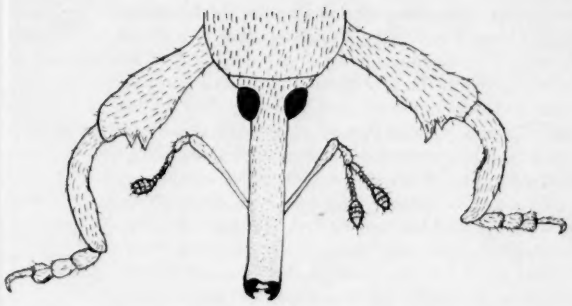

FIG. 1. A line drawing showing the abnormal antennal condition of a male boll weevil of the College Station-resistant strain.

a few genes involved (Bridges & Brehme 1944). Had this been the case, the mating of the forked-antenna male with his daughters and grand-daughters should have revealed the character in a portion of his offspring.

A genetic marker which can be successfully used in resistant studies should be associated with simple inheritance. Since this does not appear to be the case here, the feasibility of using this character as a genetic marker does not appear possible.

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A Comparison of Two Sampling Techniques for Lygus Bugs and Stink Bugs on Cotton[1]

S. R. RACE[2]

The limitations of the standard 15-inch insect sweep net as a sampling device for plant feeding and inhabiting insect species are widely recognized. Its use in obtaining an accurate index of such destructive cotton insects as lygus bugs (*Lygus lineolaris* (P. de B.), *L. hesperus* Knight and *L. elisus* Van Duzee) and stink bugs (*Chlorochroa sayi* Stål., *C. ligata* (Say), and *Thyanta custator* (F.)) is a point of particular concern.

The damage caused by these insects is often obvious, and yet apparently subeconomic numbers of these pests are collected with the nets. This is especially true during the latter half of the growing season, when the majority of plants are 3 feet tall and the sweep net will collect only those insects near the tops of the plants. Consequently, many such insects remain undetected beneath this normal sweep zone.

During July 1958, a comparison was made between cotton insect populations collected with a standard insect sweep net and with a mechanical insect collection device.[3] This study was initiated to help determine whether more accurate estimates of the existing insect populations in cotton fields could be made on the basis of sweep-net collections. As it was known that the mechanical device could collect large numbers of insects, it was assumed that it would measure the insect populations more precisely than the sweep net. A comparison of samples by the two methods taken concurrently might provide a basis for more accurate interpretation of the sweep-net method, which is more practical.

METHODS.—Four adjoining plots in a spray control experiment were selected for this study. During the collection experiment each plot except the untreated was sprayed three times with its respective insecticide. Each plot was approximately 1 acre in size and 36 rows wide, thus minimizing the small plot collection error. One hundred sweeps, each an arc of 180 degrees, were made in each plot between two rows of plants. Several minutes later the mechanical device, mounted on the front of a tractor, was run between two different rows for the same distance required to make the 100 sweeps. The tractor was run 8 to 10 rows removed from the swept rows. In effect, the mechanical device forced blasts of air simultaneously across the upper 18 inches of two rows of plants and actually blew the insects from the plants and into muslin collection sacks located on each side of the machine. The collected insects were anesthetized in the field and counted in the laboratory.

Samples were taken four times during July 1958, and many insect species were counted and compared. Results of two of the more important groups of New Mexico cotton insect pests, lygus bugs and stink bugs, the populations of which are normally determined with sweep nets, are presented in table 1.

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[2] Assistant Professor of Entomology, New Mexico Agricultural Experiment Station, University Park.

[3] The Nisbett "Bug Catcher." A. R. Nisbett and Sons, San Angelo, Texas.

Table 1.—Comparisons of total numbers of lygus bugs and stink bugs collected with a mechanical device or with a sweep net on cotton plants. University Park, New Mexico, 1958.

TREATMENT	DATES OF COLLECTION										
	July 11		July 14		July 23		July 28		AVERAGE		AVERAGE RATIO
	M.D.[a]	S.N.[b]	M.D.	S.N.	M.D.	S.N.	M.D.	S.N.	M.D.	S.N.	
Lygus Bugs (*Lygus lineolaris*, *L. hesperus* and *L. elisus*)											
Toxaphene+DDT	32	8	22	4	9	0	24	1	21.7	3.2	7:1
Toxaphene+parathion	20	3	28	13	8	2	29	1	21.2	4.2	5:1
Malathion+DDT	22	1	28	9	0	3	5	1	13.7	4.5	3:1
Untreated	90	10	87	10	—	7	12	1	63.0	9.3	7:1
Stink Bugs (*Chlorochroa sayi*, *C. ligata* and *Thyanta custator*)											
Toxaphene+DDT	17	1	42	0	24	0	9	1	23.0	0.5	46:1
Toxaphene+parathion	—	—	41	3	29	0	28	2	24.5	1.2	20:1
Malathion+DDT	10	0	41	0	22	0	11	0	21.0	0	—
Untreated	25	1	61	4	—	1	57	0	47.6	1.5	32:1

[a] Mechanical device.

[b] Sweep net.

DISCUSSION.—The data presented in table 1 clearly show that considerably more lygus bugs and stink bugs were collected with the mechanical device than with the sweep net; however, these population counts were not consistent enough to establish a predictable ratio between these two collecting methods. The average ratios of the lygus bug and stink bug populations indicate, however, their zones of habitation on cotton plants. The lygus bugs tend to inhabit the periphery of the plants, where the young squares are developing, whereas the stink bugs frequent the inner and lower portions of the plants, where the maturing bolls are located during this time of year. This difference in habitation allows for more lygus bugs than stink bugs to be collected with the sweep net.

These data indicate that sweep-net collections fail to provide a dependable population index of lygus bugs and stink bugs on cotton plants during the latter half of the growing season. This sampling method should be used only in conjunction with visual inspection of the plants in the field.

Evaluation of Insecticides for Control of the Tarnished Plant Bug on Birdsfoot Trefoil[1]

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The tarnished plant bug, *Lygus lineolaris* (P. de B.), is a major pest of birdsfoot trefoil, *Lotus corniculatus* L., grown for seed (Neunzig *et al.* 1958, MacCollom 1958, Ridgway & Gyrisco 1959). As with any destructive insect, it is necessary to determine the most practical and effective insecticides for its control. Therefore, in the summer of 1959, ten insecticides were evaluated for control of the tarnished plant bug on birdsfoot trefoil.

METHODS AND MATERIALS.—Dimethoate, Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Methyl Trithion® (*O-O*-dimethyl *S*-(*p*-chlorophenylthio) methyl phosphorodithioate), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate), Shell 5539 (phosphoric acid, dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl ester), Sevin® (1-napthyl methylcarbamate), and ethion were applied on August 2, 1959, to European birdsfoot trefoil for the control of the tarnished plant bug. Because of its proven effectiveness for tarnished plant bug control (Hopkins & Gyrisco 1951) aldrin was included also to serve as a standard. These insecticides were applied as sprays to 20- by 60-foot plots that were randomized within blocks and replicated three times. Each plot was surrounded by a 5-foot border strip to reduce contamination by spray drift. All of the insecticides were applied at the rate of 1.0 pound per acre of actual toxicant, with a truck-mounted, low pressure, low gallonage "Yellow Devil" sprayer using 40 p.s.i. of pressure and 20 gallons of spray per acre. The commercial-type sprayer had an 18-foot brush type boom equipped with 11 No. 8002 T-jet nozzles.

Twenty-five sweeps with a 15-inch diameter insect net were taken from each plot on the 2nd, 7th, and 14th day after application. From these sweepings, the adults and nymphs of the tarnished plant bug were sorted and counted. For statistical comparisons the counts for each treatment from each collection date were pooled and analyzed, using the analysis of variance. The means were then compared using Tukey's h.s.d. test at the 5% level.

RESULTS.—The combined numbers of nymphs and adults of the tarnished plant bug taken on three different sampling dates are presented in table 1. The insecticides that gave outstanding control of the tarnished plant bug over the 2-week test period were dimethoate, Dibrom, Dylox, Methyl Trithion, and aldrin. Guthion was found to be intermediate in effectiveness, while Trithion, Shell 5539, Sevin, and ethion were least satisfactory for control of the tarnished plant bug.

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[1] Accepted for publication April 4, 1960.
[2] The authors gratefully acknowledge the assistance of S. W. Jacklin in some of the field work.

Table 1.—Mean number of tarnished plant bugs from 25 sweeps and per cent control at intervals indicated after application of insecticides.

INSECTICIDE[a]	2 DAYS		7 DAYS		14 DAYS		AVERAGE ALL DAYS	
	No. Bugs	Control (%)	No. Bugs	Control (%)	No. Bugs	Control (%)	No. Bugs[b]	Control (%)[c]
Dimethoate	0.7	98	1.3	94	0.7	95	0.9	96
Dibrom	1.7	95	1.0	95	0.3	98	1.0	96
Dylox	1.3	96	2.0	91	0.3	98	1.3	94
Methyl Trithion	1.0	97	3.1	85	1.0	92	1.8	92
Aldrin	3.3	90	2.0	91	1.7	87	2.0	91
Guthion	5.0	85	5.0	77	3.1	77	4.9	78
Trithion	9.3	72	8.7	71	4.3	68	7.4	67
Shell 5539	6.7	80	12.0	59	5.7	58	8.0	65
Sevin	6.7	80	12.3	45	6.0	55	8.1	64
Ethion	16.1	52	12.3	45	5.3	60	11.6	49
Untreated	33.7	—	21.3	—	13.3	—	22.8	—

[a] All toxicants were applied at the rate of 1.0 pound per acre at Lake Ridge, New York. 1959.
[b] Insect counts were transformed, for statistical purposes, using $\sqrt{x+0.5}$, where x is the total number of nymphs and adults taken on each sampling date. All means flanked by the same line are not significantly different according to Tukey's h.s.d. test at the 5% level.
[c] Computed from preceding column.

Effect of Acceleration Force on Insect Mortality[1]

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Insects have many qualifications that recommend them for use in measuring the effects of outer space on life (Pipkin & Sullivan 1959, Sullivan & Westlake 1959, Sullivan & Smith 1960). In this study the acceleration force required to cause a 50% mortality (LG-50) was measured for several insects to determine their capability for withstanding the stress of rapid acceleration or deceleration during space flights.

The adult Japanese beetles (*Popillia japonica* Neum.) used in these experiments were collected in nature at Beltsville, Maryland, just prior to testing. The male house flies (*Musca domestica* L., 2- to 3-day-old NAIDM (National Association of Insecticide and Disinfectant Manufacturers) 1948 strain) and the adult flour beetles (*Tribolium confusum* Duv.) were reared in the laboratory. It was necessary to anesthetize the flies with carbon dioxide for handling purposes.

After completion of a test the insects were placed in a suitable container with food. Mortality counts were made in 1 day for the house fly and in 10 days for the other insects.

The insects were subjected to a force of multiple g's (earth's gravity) by placing one house fly, one flour beetle, or five Japanese beetles in each of the four tubes of a refrigerated centrifuge (22° to 24° C.) equipped with a high speed head capsule capable of applying 25,000 g's to the tubes. The tubes reached maximum speed in 90 seconds and deceleration time was approximately 3 minutes. All exposure periods are timed at indicated g's. The Japanese beetle, house fly, and flour beetle tests were repeated 7, 18, and 19 times, respectively.

The following equation was used for calculating the relative centrifugal force (R.C.F.) as related to the earth's gravity (g):

$$\text{R.C.F. (in g's)} = 0.00001118 \times r \times N^2$$

r = rotating radius (6.7 centimeters)
N = rotating speed (r.p.m.)
g = earth's gravity

In most instances the insects that were killed were dead by the end of the centrifuging period. Insects that survived centrifuging were normal in appearance. Most dead flies were eviscerated.

The data are plotted as acceleration-mortality curves in figures 1, 2 and 3. The LG-50's estimated from the curves are given in table 1.

From these results it is apparent that insects are highly resistant to the stresses imposed by extreme forces of acceleration. The small hard-shelled flour beetle required 73 minutes' time at 20,600 g's to produce a 50% mortality. The small soft house fly was less resistant but required 11,400 g's over a 2-minute period to give a 50% mortality. The relatively large hard-shelled Japanese beetle was least resistant to multiple g's of these insects

Table 1.—The LG-50 for some insects.

INSECT	MINUTES EXPOSED TO g's	LG-50
Popillia japonica	2	6,250 g's
	10	5,800
	30	4,150
Musca domestica	2	11,400
Tribolium confusum	73[a]	20,600

[a] Extrapolated slightly.

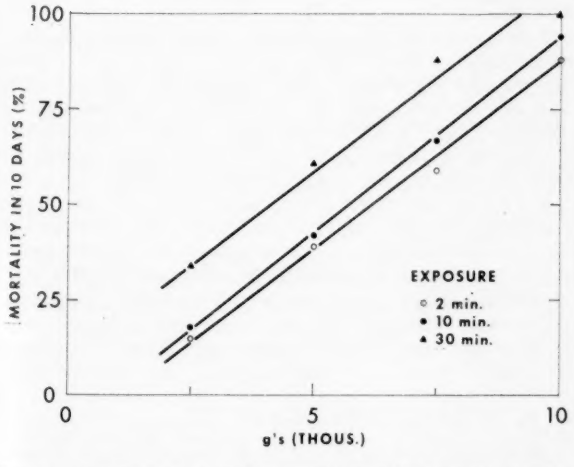

FIG. 1.—Acceleration-mortality curve for adult Japanese beetles exposed for various periods of time at indicated g's.

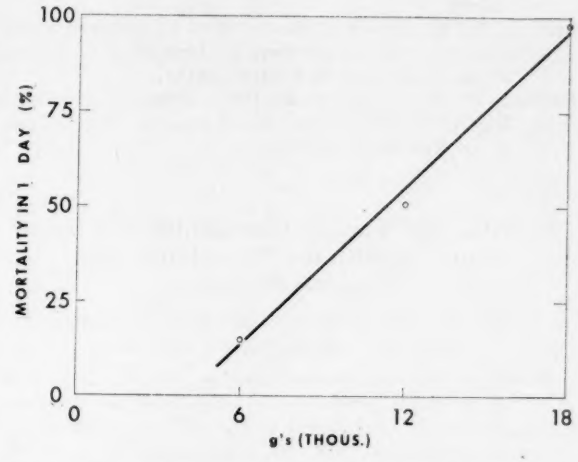

FIG. 2.—Acceleration-mortality curve for adult male house flies exposed for 2 minutes at indicated g's.

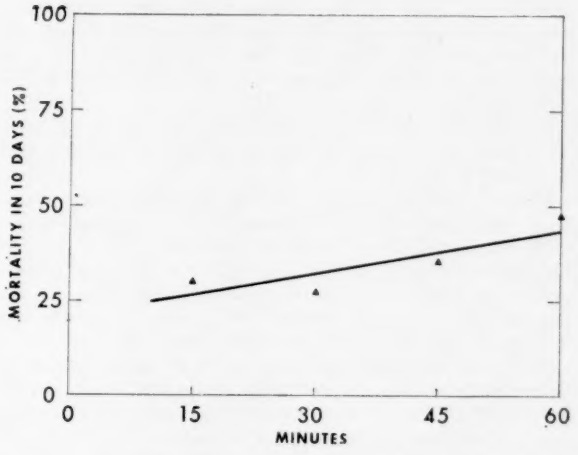

FIG. 3.—Mortality-time curve for adult confused flour beetles exposed for indicated minutes at 20,600 g's.

[1] Accepted for publication April 7, 1960.

[2] The helpful suggestions of Dr. S. R. Dutky, J. C. Keller, and W. Gersdorff are appreciated.

tested. By comparison, man has structural damage when exposed to 18 to 45 g's for short duration (Anonymous 1959).

These findings indicate that insects can tolerate any acceleration forces involved in escaping or returning to heavenly bodies.

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Systemic and Topical Insecticidal Activity of Some Substituted Phosphates and Phosphorothioates[1]

A. D. FLYNN[2] and W. G. EDEN, *Auburn University Agricultural Experiment Station, Auburn, Alabama*

Results of numerous investigations on the effectiveness of chemicals as animal systemic insecticides have been reported. Little effort has been devoted to the influence of molecular variation on animal systemic activity and virtually none to the relationship between systemic and topical activity. Hewitt *et al.* (1958) investigated a series of carbamoyl alkyl phosphorodithioates using the mouse-yellow fever mosquito host-parasite combination. In an investigation of 19 compounds dimethoate was the most active. In the structure-activity relationship there was a marked superiority of *O,O*-dimethyl compounds over corresponding diethyl. Higher *O,O*-dialkyl compounds were generally inactive at tolerated doses or were more toxic than the corresponding dimethyl compounds.

The research reported herein deals with the systemic and topical insecticidal activity of some substituted phosphates and phosphorothioates.

MATERIALS AND PROCEDURE.—Eighteen substituted phosphate and phosphorothioate compounds were evaluated for systemic insecticidal activity when administered orally to rabbits. The bed bug (*Cimex lectularius* L.), Gulf Coast tick (*Amblyomma maculatum* Koch) yellow-fever mosquito (*Aedes aegypti* (L.)), and stable fly (*Stomoxys calcitrans* (L.)) were used as the test arthropods. The LD-50's of 16 of the compounds were determined by topical application to the unengorged bed bug and 14 to the engorged adult stable fly. The compounds (table 1) were synthesized by Dr. G. M. Kosolapoff and associates of the Department of Chemistry of Auburn University except Dow ET-15 (*O*-methyl *O*-(2,4,5-trichlorophenyl) phosphoramidothioate) and VC-13 (*O,O*-diethyl *O*-2,4-dichlorophenyl phosphorothioate), which were supplied by the manufacturers.

Criteria for evaluating the systemic activity of the compounds were essentially the methods described by Adkins *et al.* (1955). The compounds were diluted with acetone and administered as single oral doses to rabbits. The number of test arthropods that fed on each rabbit varied from 6 to 37; tests were unreplicated. The toxicants were applied topically to the abdomen of the bed bug and stable fly with the micrometer-syringe device described by Owen & Haynes (1958). Ten insects were used per treatment; treatments were replicated 3 to 10 times. The method of Litchfield & Wilcoxon (1949) was used to construct the dosage-mortality curves.

RESULTS.—The approximate highest sublethal dose to the rabbit reflected the relative toxicity of the compounds (table 1). The most toxic material was *O*-methyl, *O*-ethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate (highest sublethal dose of 50 mg./kg.) followed by *O,O,O*-trimethyl phosphorothioate (75 mg./kg.). The substituted phenyl phosphates were more toxic than the corresponding phosphorothioates. The least toxic compound to the rabbit was *O,O*-dimethyl *O*-(phenyl) phosphorothioate. The addition of chlorine to the *O,O*-dimethyl *O*-(phenyl) compounds increased mammalian toxicity. One chlorine atom in the 2- or 4-position of the phenyl ring increased toxicity slightly in the *O*- and *S*-analogs but position was of no consequence. The dichloro-substituted compounds were more toxic than the mono-substituted ones, and the tri-'s were more toxic than the dichloro substitutions. In the 2,4,5-trichloro phosphorothioates, replacement of the CH_3O groups with NH_2 lowered toxicity, while replacement of one CH_3O with C_2H_5O greatly increased toxicity to the rabbit.

The only compounds that exhibited systemic activity against the bed bug contained dimethyl or methyl ethyl groups with di- or tri-Cl substitutions in the phenyl ring. In the dimethyl compounds the 2,4,5-trichloro substituted *O*- and *S*-analogs were highly systemic, but the 2,4,6-trichloro *O*-analog was not active systemically. The failure of the 2,4,6-Cl compound as a systemic indicated significance of position of one Cl atom in the trichloro-substituted compounds. Replacement of one CH_3O group with a C_2H_5O in the 2,4,5-Cl *S*-analogs resulted in a highly systemic insecticide against the bed bug. In fact, the methyl to ethyl substitution may have increased activity, since the dosage to the rabbit was less. Replacement of one or both CH_3O groups in the 2,4,5-Cl phosphorothioates with NH_2 completely destroyed systemic activity against the bed bug. In the dimethyl di-Cl substituted compounds only the 2,4-Cl *O*-analog was systemic against the bed bug. The 2,5-Cl *O*-analog was not systemic, which showed the significance of position of the Cl atom. Inasmuch as the 2,4-Cl *S*-analogs of both the dimethyl and diethyl compounds were ineffective against the bed bug, differences in systemic activity between the phosphates and phosphorothioates were indicated.

The *O*- and *S*-analogs of the 2,4-Cl substituted dimethyl compounds were effective against the Gulf Coast tick. The 2,5-Cl *O*-analog was ineffective against the tick as well as the bed bug. All of the dimethyl tri-Cl compounds were ineffective, but the methyl-ethyl tri-Cl *S*-analog was systemic against the Gulf Coast tick.

The only compound that was highly systemic against the yellow-fever mosquito was *O,O*-dimethyl *O*-(2,4-dichlorophenyl) phosphate. The methyl-amino tri-Cl *S*-analog and the *O,O,O*-trimethyl phosphate, though showing some activity, were less effective.

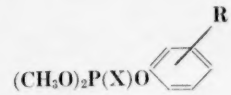
Both the *O*- and *S*-analogs of the 2-Cl substituted dimethyl compounds were highly effective systemically against the stable fly. Only the *O*-analog of the 4-Cl compounds was active, suggesting a relationship between Cl-substitution and the systemic activity of the phosphorothioates. The 2,4- and 2,5-dichloro substituted dimethyl compounds were highly systemic against the stable fly, but the corresponding diethyl *S*-analog was low in activity. The 2,4,5-trichloro dimethyl compounds were highly effective systemically, but the corresponding 2,4,6-trichloro analog was much less active. The methyl-amino 2,4,5-Cl *S*-analog was also highly active systemically against the stable fly.

It is interesting to note that only one compound, *O,O*-dimethyl *O*-(2,4-dichlorophenyl) phosphate, was highly systemic against the four test arthropods. In general, the results of this study

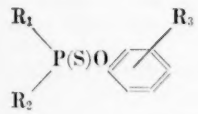
[1] The data presented herein were taken from a thesis presented to the Graduate School of Auburn University in partial fulfillment of the requirements for the Ph.D. degree. The investigation was supported in part by a research grant, PHS G-4065 (c) from Division of Research Grants of the National Institutes of Health, Public Health Service. Accepted for publication April 11, 1960.

[2] Present address: U. S. Public Health Service, Savannah, Georgia.

Table 1.—Relative systemic and topical insecticidal activity of some substituted phosphates and phosphorothioates.



X	R	Dosage to Rabbit (Mg./Kg.)	Systemic Activity: Per Cent Mortality[a] Bed Bug 24 Hr.	Gulf Coast Tick (After Molting)	Yellow-Fever Mosquito 48 Hr.	Stable Fly 48 Hr.	Topical LD-50, μg./Insect: Bed Bug	Stable Fly
O	—	275				0 (7)		
		300	0 (20)	6 (17)	0 (7)		31.00	6.63
S	—	600		15 (31)		27 (15)		
		675	10 (10)				10.13	
O	2-Cl	250	0 (15)	33 (24)	0 (6)	100 (13)	4.87	0.01
S	2-Cl	400		5 (21)	0 (12)	100 (15)		
		425	0 (12)				113.20	1.38
O	4-Cl	250	0 (19)	21 (33)		93 (15)		
		275			8 (13)		13.45	0.55
S	4-Cl	425	0 (15)	0 (7)	0 (24)	21 (14)	71.50	10.25
O	2,4-Di-Cl	200	94 (17)	92 (36)	100 (19)	100 (10)	0.43	0.02
S	2,4-Di-Cl	430	13 (88)	76 (21)	0 (6)	100 (17)		
O	2,5-Di-Cl	225		0 (23)		100 (20)		
		240	0 (12)					
		275			29 (7)		0.83	0.15
O	2,4,5-Tri-Cl	150	100 (25)	5 (37)	17 (23)	100 (10)	0.04	0.01
S	2,4,5-Tri-Cl[b]	200	100 (10)	23 (13)	0 (12)	100 (20)	0.03	0.03
O	2,4,6-Tri-Cl	200	0 (20)	0 (32)		33 (16)		
		225			8 (13)		4.47	2.38



R_1	R_2	R_3						
C_2H_5O	C_2H_5O	2,4-Di-Cl[c]	100	0	7 (15)	4 (26)	40 (10)	3.44	0.05
CH_3O	C_2H_5O	2,4,5-Tri-Cl	50	100 (20)	73 (15)	0 (37)	0 (12)	0.08	0.05
CH_3O	NH_2	2,4,5-Tri-Cl[b]	300	0 (25)	5 (21)	64 (14)	100 (11)	0.05	0.02
NH_2	NH_2	2,4,5-Tri-Cl	350	0 (15)	0 (7)	0 (8)	0 (14)		
		$(CH_3O)_3P(O)$	425	0 (15)	32 (19)			
			450			80 (26)		146.00	238.04
		$(CH_3O)_3P(S)$	75	0 (15)	3 (26)	0 (17)	0 (8)	31.50	29.20

[a] Numbers in parentheses refer to the number of insects that fed on the host.
[b] Technical material provided by Dow Chemical Company.
[c] Technical material provided by Virginia-Carolina Chemical Company.

indicated that the most active systemic compounds were *O,O*-dimethyl *O*-phenyl phosphates with di- or tri-Cl ring substitutions in the 2,4- or 2,4,5-positions. The compounds that were the most active systemically against the bed bug and stable fly were also the most toxic to both species when applied topically.

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Introduction and Establishment of *Anagyrus antoninae* on Rhodes-Grass Scale in Mexico[1]

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Rhodes-grass scale, *Antonina graminis* (Maskell), was first recorded in Mexico by the Officina de Defensa Agricola from Ciudad Valles, San Luis Potosi, Mexico, on December 3, 1956. The scale insect was reported infesting more than 300,000 acres of pasture grasses in the states of Vera Cruz, Tamaulipas, and San Luis Potosi following a survey during 1957. Additional reports from other infested areas showed greater distribution of the scale insect.

Early host records of the scale in Mexico showed Para grass as one of great importance. Mexican authorities reported that Para grass is one of the most important grasses in Mexico for livestock. Para grass was found to be one of the most heavily attacked grasses by Rhodes-grass scale, although Chada & Wood (1960) did not list this grass as one of the important species that is heavily attacked by the scale. Para grass is commonly used on canal and lake banks for conservation of banks in the Lower Rio Grande Valley area and is heavily attacked by the scale during much of the year.

Officials of the Departmente de Control Biologico began efforts to obtain biological controlling agents to combat this problem. Permits were issued by the Director General de Defensa Agricola and the Insect Identification and Parasite Introduction Research Branch of U. S. Department of Agriculture to introduce the parasite, *Anagyrus antoninae* Timberlake, into Mexico. Dean & Schuster (1958) reported the greatest extent of parasitism occurred during October to March in the Lower Rio Grande Valley area whereas parasite activity was lowest during the summer months. Parasite activity appeared to be greatly reduced when temperatures remained above 90° F. several hours per day. Such high temperatures appeared to be the main factor contributing to the failure of the parasite to become established on King Ranch. However, certain areas of Mexico were known to have continued high relative humidity and mild temperatures throughout the year. These areas offered the best potential climatic zones for establishment of the parasite.

Introductory shipments were made of rearings from parasitized scale on field-collected Para grass. Air mail shipments of adult parasites were made to Tampico and Micos. A customs broker from Reynosa picked up the shipments to Micos and placed them on the plane. A biological control worker met the plane at Tamuin and carried the parasites to Rancho Don Tomas at Micos some 12 miles west of Valles. This 10,000-acre ranch was planted principally to Para grass. The author carried 350 parasites by airplane to this ranch during April 1958, and 21 by automobile during July 1958. On a visit to the ranch on March 25, 1959, the following parasites were released: 7 female and 8 male *A. antoninae*, 17 female and 9 male *Anagyrus diversicornis* Mercet, and 12 *Timberlakia europaea* (Mercet), the latter two parasites reared from *Antonina purpurea* Sign. in quarantined shipments from France. Shipments are summarized in table 1.

One male emerged from field-collected material during July 1958. Both females and males emerged from this material after the manager of the ranch carried the material back to Mexico City with him for the first recovery of the parasite under field conditions.

Table 1.—Shipments of adult *Anagyrus antoninae* to Mexico.

DATE	SHIPMENT NO.	NO. OF PARASITES	DESTINATION
December 1957	5	612	Tampico, Tamaulipas
March 1958	4	569	Micos, San Luis Potosi
April 1958	2	521	Micos, San Luis Potosi
July 1958	1	21	Micos, San Luis Potosi

Table 2.—Parasitism of *Antonina graminis*[a] by *Anagyrus antoninae* at Micos, Mexico, during March 1959.

LOCATION	PARASITISM (%)	PARASITES PER PARASITIZED SCALE	DEGREE OF SCALE INFESTATION
1	1.5	1.3	Medium
2	2.0	1.5	Low
3	0.0	0.0	Very low
4	2.5	2.4	Very low
5	41.5	2.8	Very low

[a] Two hundred scales examined at each location.

A trip was made to the ranch during the last week of March 1959 after several requests were received at Weslaco for additional parasite material. Five locations were selected for collection of scale-infested Para grass to determine the extent of parasitism. Valley floors around Micos were very uniform in grade and mountains were approximately 400 to 1,750 yards apart, 1,200 feet above sea level. Collection sites varied from valley floors where temperatures remained hot over a longer period of the day to the side of a mountainous waterfall where relative humidity was continually high and where the mountain shaded the area from the sun during half of the day, resulting in lower temperatures. From June 1958 to March 1959, the humid conditions kept the grass in very good condition. Later in 1959, an extended drouth occurred and reports were received that high infestations of Rhodes-grass scale were present on several areas of the ranch. The extent of parasitism was determined by microscopic dissection of scale for parasite larvae and pupae. The results are shown in table 2.

The first three locations were in the open valley between mountains and location 4 near the mountain side. Location 5 was at the waterfall, previously described, and had an unusually high percentage of parasitism with a higher number of parasites per parasitized scale than at the other locations. An unusually large number of near-mature parasite larvae was found in the dissections.

DISCUSSION.—*Anagyrus antoninae* was found established on *Antonina graminis* attacking Para grass at Micos S.L.P., Mexico, 1 year after the parasite was introduced from Weslaco, Texas. Parasitism was very high under continuously high relative humidity and lower temperature conditions whereas parasitism was very low where the hotter daytime temperatures prevailed.

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[1] Investigations supported in part by Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. Technical Contribution No. 3441. Accepted for publication April 11, 1960.

Insecticides Change the Importance of Segments of Insect Generations[1]

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Regular applications of certain insecticides within narrow time periods year after year cause changes in the relative importance

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of segments of insect generations. For entomologists who work on the control of insect pests of specific crops, this phenomenon may be second in importance only to the problem of insect resistance to pesticides.

For years in southern Pennsylvania, the petal fall and first cover (10-day) sprays were important in the control of the codling moth, *Carpocapsa pomonella* (L.). Apples on check trees not treated with lead arsenate during this period became infested with codling moth larvae before application of the second cover spray. Then, in the early 1950's, growers began using organic thiophosphate insecticides in the petal fall and first cover sprays. These pesticides are toxic to the adults, larvae, and, in some cases, the eggs of the codling moth. Nowadays, it is rare to find an apple infested with an early first generation codling moth larva on trees not treated with the petal fall and first cover sprays. On the other hand, apples not treated to protect them from the last segment of the first generation of this pest (that segment of the generation formerly neglected by growers) usually become heavily infested. This tail-end portion of the first generation of the codling moth has become so important in southern Pennsylvania that a spray timed to catch it has to be included in the spray schedule.

A similar shift in importance has occurred in the case of the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.). For a number of years, sprays pinpointed to hit the peaks of each of the three annual generations successfully controlled this pest. The so-called "stragglers" at the end of each generation were regarded as a minor nuisance. Gradually, the "stragglers" have become numerically important and the spray schedules have to be designed to deal with them as well as with earlier segments of the generations.

The increase in the importance of the "stragglers" at the tail-end of generations of the codling moth and of the red-banded leaf roller occurred only after highly toxic insecticides had been employed within fairly narrow time periods for several successive years. Is this not a case of selection through the use of insecticides? Such phenomena illustrate the importance of close observation of insect pests, the necessity for realizing that the whole span of an insect's life cycle should be considered in connection with plans for its control. Knowing that a chemical is toxic to a particular insect and that it may be used to control that pest is by no means all the information required to employ the chemical successfully to protect a specific crop from injury by the pest over a period of years.

Most entomologists will grant that the regular use of an insecticide will eventually select strains of insects resistant to that insecticide. It seems that the regular application of insecticides may be the cause of selection for other characteristics.

Control of the Corn Earworm with American Cyanamid 18133[1]

R. A. BLANCHARD,[2] LOUIS W. GETZIN,[3] and W. A. DOUGLAS[2]

American Cyanamid 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) has been tested largely against nematodes and soil insects, but has also shown promise for the control of insects that feed above ground. This chemical was included in six small-scale experiments conducted in 1959 for control of the corn earworm (*Heliothis zea* (Boddie)) in sweet corn. It was tested in 10% granules at Weslaco, Texas, and State College, Mississippi, as well as in 7.5% granules and in sprays made from the 45% emulsifiable concentrate at State College. The granules varied in size from dustlike particles to those resembling coarse sawdust. The Texas tests were carried out during May and those in Mississippi during June, July, and August.

Plots were single rows 33 feet in length replicated four times in all but one test at Weslaco, which was unreplicated, and one test at State College, which had three replications. In the unreplicated test with granules, silks on marked ears were treated one, two, and three times beginning the second day after they were extruded. All other applications were begun the first day silks appeared in the plots and made every third day. Standard sprays containing DDT with and without mineral oil were included in four of the tests.

Table 1.—Control of the corn earworm in sweet corn with American Cyanamid 18133 and DDT.

Test No.	Time	Treatment[a]	No. of Applications	Worm-Free Ears (%)
		Weslaco, Tex.		
1	May	Am. Cyanamid 18133 (10% granules)	3	95
		DDT (emulsion)		
		1½ lbs.+1½ gal. mineral oil	3	92
		2 lbs. (no oil)	3	96
		Untreated	—	7
2	May	Am. Cyanamid 18133 (10% granules)	1	38
			2	100
			3	100
		Untreated	—	0
		State College, Miss.		
1	Mid-June to early July	Am. Cyanamid 18133 (10% granules)	3	100
		DDT (emulsion)		
		2 lbs.+1½ gal. mineral oil	4	79
		2 lbs. (no oil)	4	70
		Untreated	—	0
2	Mid-June to early July	Am. Cyanamid 18133 (10% granules)	3	100
		DDT (emulsion)		
		2 lbs.+1½ gal. mineral oil	3	68
		2 lbs. (no oil)	3	51
		Untreated	—	0
3	Mid-July	Am. Cyanamid 18133 (7.5% granules)	1	47
			3	57
			4	95
		Am. Cyanamid 18133 (emulsion)		
		1 lb.+1½ gal. mineral oil	4	80
		1 lb. (no oil)	4	78
		Untreated	—	22
4	Late July to early August	Am. Cyanamid 18133 (10% granules)		
		Small	4	39
		Intermediate	4	26
		Large	4	21
		DDT (emulsion)		
		2 lbs. (no oil)	4	20
		Untreated	—	0

[a] All DDT sprays in 25 gallons per acre.

The granules were shaken onto corn silks from a jar with holes in the lid. No attempt was made to separate the different-sized granules, except in one test. From one to four applications were made. The amounts applied per acre were not known, but all silks were covered thoroughly at each application.

The sprays were applied at the rate of 25 gallons per acre from a knapsack sprayer at pressures between 55 and 70 pounds, with a single flat fan nozzle aimed at the silk zone of the ears from each side of the row. Emulsions containing 1 pound of American Cyanamid 18133 with and without mineral oil were compared.

Results of tests are summarized in table 1.

In Texas three applications of 10% American Cyanamid 18133 granules gave excellent control of the corn earworm, and were equal to the standard DDT emulsion. Individual silk masses in this and later tests may have received only one or two applications, since silks were not all extruded at the time of the first two applications. In a second test where individual ears were marked at the time granules were applied, 38% of the silks receiving a single application remained worm-free, and two and three applications to the silks gave 100% worm-free ears.

In two tests carried out at State College between mid-June and early July three applications of 10% granules gave 100% worm-free ears and were superior to four applications of the standard DDT emulsion. In a test conducted in mid-July, one and three

[1] Accepted for publication April 15, 1960. In cooperation with the Mississippi and Texas Agricultural Experiment Stations.

[2] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] Texas Agricultural Experiment Station, Weslaco.

applications of 7.5% granules gave poor earworm control, but four applications gave excellent results. Four applications of American Cyanamid 18133 emulsion did not appear equal to four applications of granules, but in some of the plots adverse weather made it impossible to determine whether or not there was a statistical difference. In late July and early August under adverse growing conditions for sweet corn, neither the American Cyanamid 18133 granules nor the DDT emulsion gave good control. The granules had been separated into three sizes in this test, and the smallest size gave the best control.

Tests with Young Poultry for the Control of House Fly Larvae Under Caged Laying Hens[1]

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Raisers of caged poultry in central Florida have used organophosphorus larvicides for control of the house fly (*Musca domestica* L.), but within recent years resistance to these compounds has become so widespread that other remedial measures are necessary. Rodriguez & Riehl (1959) showed that house fly infestations in California poultry houses could be controlled by giving young cockerels access to the droppings in which the larvae were developing.

To determine whether this technique was applicable to Florida conditions, 2-week-old chicks of undetermined sex were confined underneath caged laying hens at the rate of one chick per 10 layers. All droppings were removed from the area a short time before the chicks were introduced, but at the time of their release the manure was 1 to 3 inches deep. In the first test the entire house was fenced and the chicks were allowed access to the complete floor area. However, because of excessive droppings in the aisles, in the second test they were confined by wire fencing to the area immediately beneath the cages. Larval density was determined by collecting a large spoonful of manure from each of 10 locations where the infestation appeared heaviest, spreading it on a plywood board, and counting the larvae. Initial counts averaged 350 in the first test and more than 1,000 in the second. All the larvae were eliminated at the end of 1 week, and the control remained complete for 5 weeks in the first test and for 2 weeks in the second. The tests were then discontinued because the poultry houses were sold to make way for a residential subdivision.

In two additional tests 1-month-old chicks were confined to the floor area directly underneath the caged layers. Untreated checks were maintained to provide comparative data on normal fly-breeding fluctuations. Within 5 days 100% control was achieved, and control remained at this level for 2 months. The tests were then terminated because no larvae were found in the untreated check area owing to the advent of winter. In all tests supplementary feed and water were made available to the chicks and no mortality was observed in any test.

Samples of the chickens eaten at the conclusion of the experiment compared favorably with those of chickens fed on mash. Application of this method transformed fly control from a costly procedure into a minor asset, and eliminated resistance, residue, and toxicity problems.

The promising results indicate the desirability of further study, particularly with 2- to 4-week-old pullets, to determine whether these fowl can be utilized for egg production upon termination of the control phase, and also because reports indicate that pullets cause less excitement among the laying flock than cockerels.

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[1] Accepted for publication April 21, 1960.

A Comparison of Two Methods of Mass-Marking Foraging Honey Bees[1]

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The use of a genetically marked strain of honey bees (*Apis mellifera* L.) called cordovan to identify experimental bees has been reported several times. Peer & Farrar (1956) and Taber (1954) used cordovans to study the mating behavior of honey bees, and Levin (1959) used this recessive mutant to study the foraging ranges of field bees. In preparation for studies on foraging ranges in Utah, the author mass-marked black Caucasian bees at the hive by tagging them with radioactive phosphorus (P^{32}) in order to find out if both techniques could be used in the same experiments.

The bees were tagged by feeding the colonies sugar sirup containing radioactive phosphorus. The 10 millicuries (mc.) of phosphoric acid solution from the Oak Ridge National Laboratory were diluted to 10 ml. with distilled water. One ml. of this dilution was added to 100 ml. of sugar sirup for each of 10 colonies and fed in Boardman hive-entrance feeders to prevent access by bees from other colonies. Because of the 14-day half-life of P^{32}, the original 10 mc. had decayed to such a degree that each colony was fed only 0.8 to 0.9 mc. of activity.

Natural nectar was so abundant at the time of feeding that the bees were slow to take up the sirup. Therefore, it was sprinkled on the tops of the frames inside each colony. No effort was made to measure the radioactivity of individual bees. Because of the natural transmission of food throughout the colony (Nixon & Ribbands 1952), 24 hours after the combs were sprinkled almost all bees captured at the hive entrances proved radioactive when monitored with a Geiger-Müller counter.

Bees collected in the field were assayed for radioactivity by two methods. The more radioactive bees were detected with the Geiger counter. Radio-autograms of the remaining bees, prepared

Table 1.—Percentages of radioactive and cordovan honey bees collected at different distances from the experimental apiary.[a]

DISTANCE	DATE OF COLLECTION (AUGUST)					
	13	14	15	16	17	21
	Radioactive Bees					
100 yards E	17.2	37.5	35.8	37.0	30.9	45.8
N	9.9	17.3	18.4	32.9	29.3	31.3
200 yards E	8.6	27.3	25.8	26.5	25.0	35.6
N	5.2	15.2	18.9	12.7	17.1	18.1
300 yards E	6.1	10.2	18.1	27.1	18.2	17.5
N	2.1	2.8	7.6	9.2	4.5	10.1
500 yards S	22.9	39.7	51.6	47.4	36.1	50.0
1,200 yards NE	0.6	6.5	0.9	3.6	2.0	3.8
NW	3.7	4.5	9.6	8.5	2.3	0
1,760 yards E	0	0	0	0.9	0	0
1.6 miles N	2.4	0	0	0	0	0
NE	0	0	1.03	0	1.0	0
	Cordovan Bees					
100 yards E	13.1	12.5	11.3	3.7	12.7	22.9
N	14.5	28.4	27.6	13.7	18.7	22.4
200 yards E	7.2	16.4	9.7	14.3	5.4	13.3
N	4.8	12.5	16.2	12.7	15.7	18.1
300 yards E	3.9	10.2	16.4	10.4	7.3	14.0
N	0.7	0	0	0	1.5	10.1
500 yards S	12.0	11.8	10.9	14.0	8.2	6.2
1,200 yards NE	0	2.6	0	0	1.0	1.2
NW	0	13.4	4.2	3.2	2.3	0
1,760 yards E	0	0	0	0.9	0	0
1.6 miles N	0	0	0	0	0	0
NE	0	0	0	0	0	0

[a] Colonies were moved to the site on August 13, 1956.

[1] Accepted for publication April 25, 1960.

[2] In cooperation with the Utah Agricultural Experiment Station. W. P. Nye and R. A. Nielsen assisted in carrying out some of the field work.

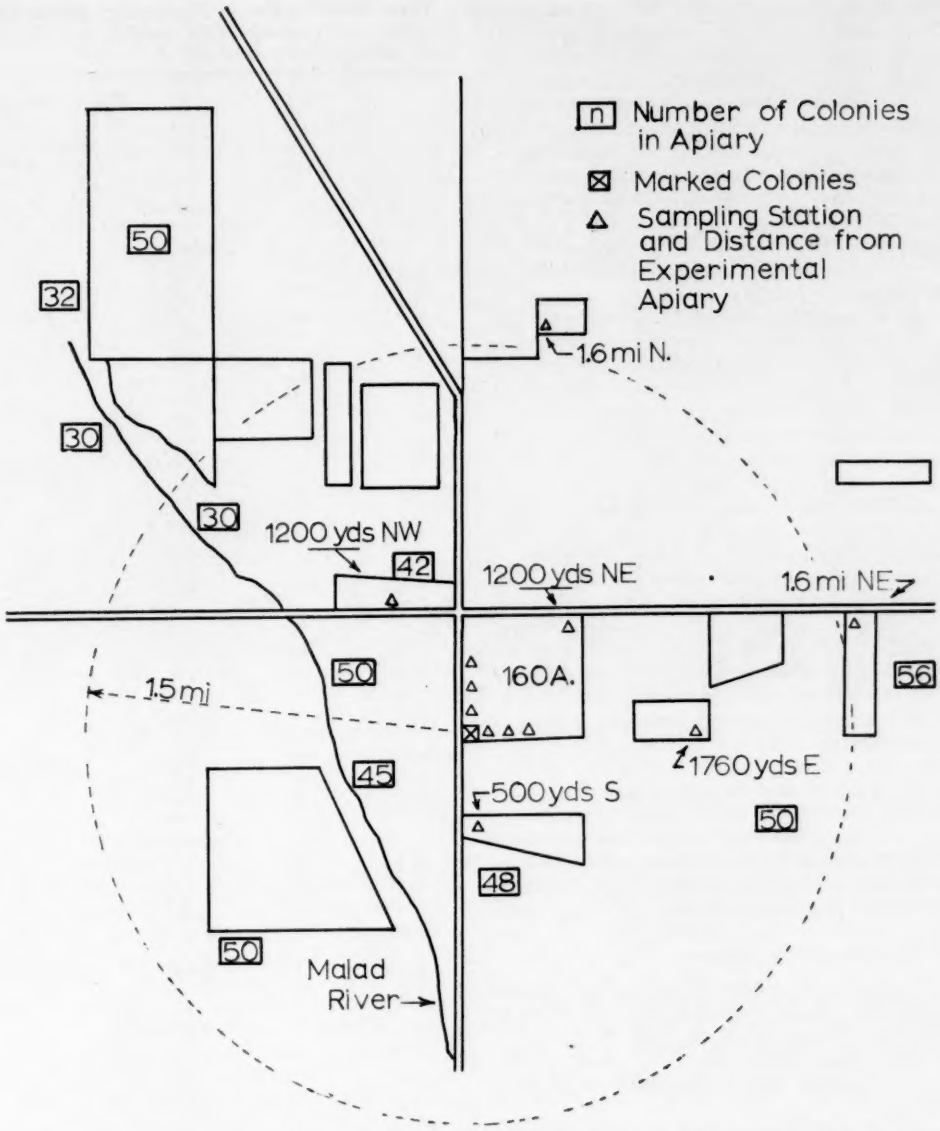

FIG. 1.—Sampling stations, alfalfa fields, and other apiaries around marked colonies, Plymouth, Utah, 1956.

by placing them on film for several weeks' contact before development, revealed other tagged individuals.

The treated colonies were isolated after the test was finished and not disturbed until the following spring, when the radioactivity had worn off completely.

The comparison was made at Plymouth, Utah, about 30 miles west of Logan, where the 10 colonies of bees that had been fed radioactive sirup and the 10 cordovan colonies were placed at the southwest corner of a 160-acre field of second-crop seed alfalfa in peak bloom. Within a 1½-mile radius of the bees was a number of fields containing about 700 acres of alfalfa, most of which was past peak bloom, and about 315 colonies in seven apiaries in addition to the experimental bees.

Sampling stations were established at 100, 200, and 300 yards in two directions within the field and at several other locations and directions beyond, as indicated in figure 1.

At each station from 25 to 150 foraging honey bees were collected with an insect net each day for 5 days, and again 4 days later. The data are presented in table 1. In general the two types of bees behaved similarly with respect to distance. The bees were killed and examined to establish the percentage that came from the experimental colonies. The area covered at each station was approximately 25 to 50 square yards, and the fluctuation in number of bees collected was caused in part by variations in populations at the stations.

The radioactive bees made up a higher proportion of most samples than the cordovans. The percentages of both types of bees became smaller as the distance from the apiary increased. As the sampling period progressed, the marked bees made up an increasing share of the bees collected at all stations.

Out of a total of 6,880 bees collected, 429 (6.2%) were cordovan and 735 (10.7%) were radioactive. In spite of this difference, both types of bees seemed to show similar gradations in population as the distance increased. The black colonies appeared to be a little stronger than the cordovans, and it is probable that this fact was responsible for the difference in the numbers recovered although no detailed observations were made on the relative strengths of the colonies.

The radioactive bees made up a larger percentage of the bees collected from the east locations than they did from the north stations. It is possible that the alfalfa was more attractive at these locations, and that the radioactive colonies were oriented

to the more favorable location more quickly than were the cordovan colonies. Unfortunately, no data were collected to support this conjecture. Also it is possible that the different prior locations of each group could have influenced their orientation.

Studies which require the identification of field bees coming from experimental colonies can probably utilize either or both methods of mass-marking without affecting the results. An attempt should be made to minimize variability in population between the groups of colonies marked by each technique, and cognizance should be taken of their original locations.

If only one type of marking is needed, the use of cordovans is recommended where they are obtainable. Their characteristic coloration does not seem to cause any genetic bias to appear. A drawback to their use lies in the possibility that after a few seasons the cordovan color may spread from experimental apiaries into commercial colonies in the area. Commercial hybrid honey bees are another source from which cordovan genes may be distributed into the natural population. For this reason the experimental fields and nearby apiaries should be surveyed before the tests begin to determine whether cordovan bees are present in other colonies.

The use of radioactivity as a marker requires equipment and facilities not readily accessible to all investigators, and it removes the treated colonies from normal production operations for the rest of the season. However, it has the advantage of being applicable immediately for the experiment with any colonies available.

The only difficulty encountered in identifying tagged bees during several seasons' work stemmed from temporary contamination of all bees and vegetation in the experimental fields by radioactive fallout from nuclear test explosions in Nevada. However, this complication is not one to be met with often, or in many places.

Several materials could be used for tagging bees. Phosphorus was chosen because of reports in the literature of its relative harmlessness, its convenient half-life of 14 days, and its emission of beta particles. Courtois & Lecomte (1958) used radioactive gold (Au^{198}) for similar studies. This material, with its half-life of 2.7 days, would not last long enough for some purposes.

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Response of Quiescent Khapra Beetle Larvae to Fumigation and to Low Temperatures[1]

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The khapra beetle (*Trogoderma granarium* Everts) may, under favorable conditions, complete its life cycle, egg to adult, in a relatively short time, *i.e.*, 30 days at 90° F. (Lindgren *et al.* 1955). Some khapra beetle larvae, however, under certain conditions such as crowding, lack of food, or the presence of contaminated (faecal pellets, shed skins, etc.) and depleted food, will delay pupation and remain in a more or less quiescent state for a long period of time at favorable developmental temperatures. Information from the Pest Infestation Laboratory at Slough Bucks (Anonymous 1958) indicates that these dormant or quiescent larvae may be resistant to fumigation. Burges (1959) discusses the occurrence of diapause larvae at a constant temperature and their behavior.

Table 1.—Results of fumigating active and quiescent khapra beetle larvae with methyl bromide, hydrocyanic acid, and acrylonitrile at 70° F.

Dosage (Lb./1000 Cu. Ft.)	Exposure (Hrs.)	Per Cent Kill		
		Active Larvae	Quiescent Larvae Held for 2 Months at:	
			90° F.	60° F.
		Methyl bromide		
2	2	29.3	0	0
3	2	66.9	0	0
4	2	87.5	1.5	2.0
5	2	92.3	6.4	13.6
6	2	98.0	6.2	13.5
2	4	88.2	2.0	2.5
3	4	100	2.0	1.8
4	4	100	18.8	71.8
5	4	100	81.6	77.8
6	4	100	77.0	86.6
1	8	77.1	0	2.4
1.5	8	100	2.0	32.6
2	8	100	11.9	91.7
2.5	8	100	81.0	98.4
3	8	100	84.9	99.1
0.5	24	100	25.0	43.5
0.75	24	100	61.8	95.2
1.0	24	100	79.1	97.4
1.5	24	100	100	100
0.375	48	100	19.6	43.4
0.5	48	100	57.5	100
0.75	48	100	100	100
		Hydrocyanic acid		
0.5	2	93.1	60.4	43.9
0.62	2	90.4	80.2	67.9
0.75	2	95.3	89.8	65.9
1.0	2	97.8	96.4	84.6
0.25	8	100	100	100
0.5	8	100	100	100
		Acrylonitrile		
0.75	2	79.1	3.5	4.1
1.0	2	100	3.6	4.2
1.25	2	100	9.9	8.3
0.5	4	91.3	4.0	3.9
0.75	4	100	17.8	9.1
1.0	4	100	20.9	15.6
0.25	8	7.8	4.2	4.4
0.5	8	100	17.4	30.7
0.75	8	100	31.8	26.8

The work discussed in this paper was conducted in the laboratory and describes a simple method of obtaining quiescent khapra beetle larvae and the response of these larvae to fumigation and to low temperatures. Burges (1959) obtained quiescent larvae by clipping together two blocks of wood 4.5×7.5×0.9 cm. with elastic bands and held apart along one side only by a narrow strip of cardboard. A crevice 1.2 mm. in width seemed to be optimum. In the work reported here, two transparent synthetic plastic plates 1/16×2×2 inches were used. A small piece of

[1] Accepted for publication April 15, 1960.

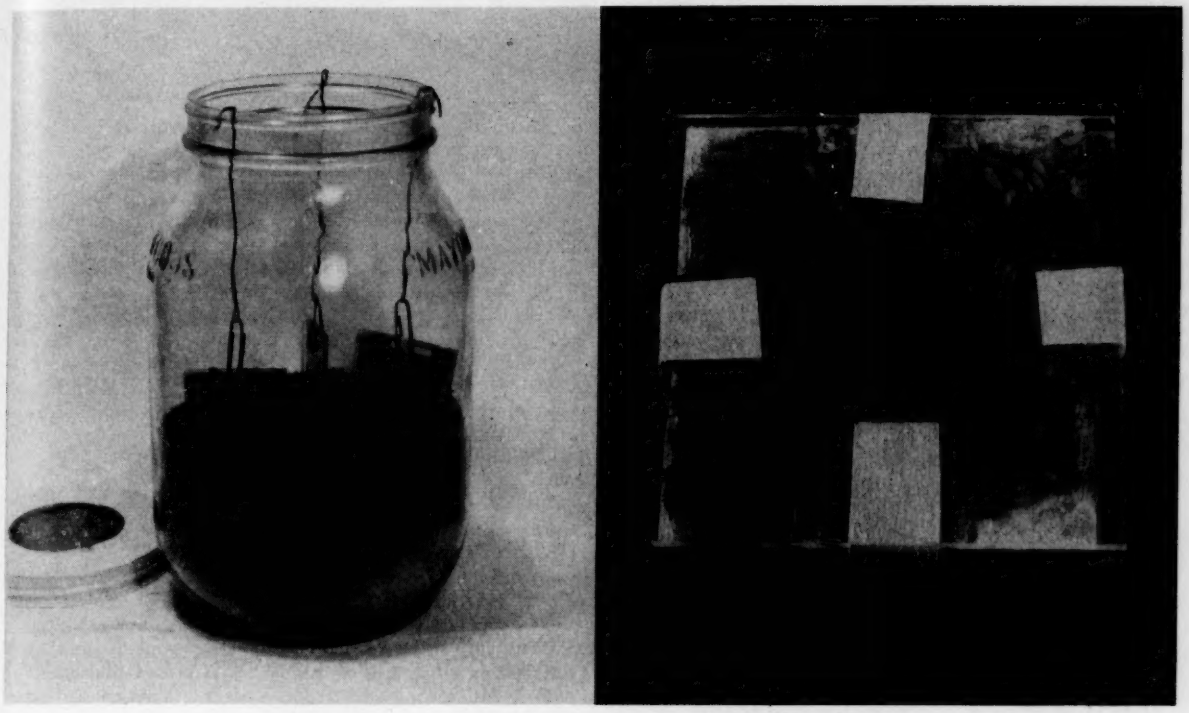

FIG. 1.—Left: Hibernating cells suspended in khapra beetle culture. Right: Hibernating cell containing quiescent khapra beetle larvae.

cardboard of the proper thickness (approximately 1/16 inch) was placed between two of these plastic plates along with a piece of paper towelling 2×2 inches. The plastic plates with the cardboard spacers and paper towelling between were secured with masking tape (fig. 1, right). Three to four of these cells were suspended in gallon jars containing khapra beetle cultures with the lower edge of the cell being submerged about ¼ inch below the surface of the culture (fig. 1, left). Khapra beetle larvae snugly packed the crevice on one side of the paper towelling. Only occasionally would a few be found on the other side. These hibernating cells were changed twice weekly and were placed in petri dishes, some being held at 60° F. and some at 90° F. for further experimentation. To obtain large numbers of quiescent larvae, the cultures held at 90° F. were kept crowded and no fresh food was added, the food present being contaminated and depleted. The advantages of transparent hibernating cells are obvious.

Studies being made on factors causing larvae to enter and to leave these hibernating crevices, loss of weight of quiescent larvae, number of moults, et cetera, will be reported in a later paper.

RESPONSE TO FUMIGATION.—Results of experiments comparing the susceptibility of active and quiescent khapra beetle larvae to methyl bromide, hydrocyanic acid, and acrylonitrile are given in table 1. Fumigations were conducted in 100-cu. ft. chambers under atmospheric pressure and at a temperature of 70° F. Active and quiescent larvae were exposed together and all experiments were replicated. The quiescent larvae used in the fumigation experiments were held at 60° or 90° F. for approximately 2 months from the time they entered the crevice in the cell until they were fumigated. The active larvae were obtained from cultures held at 90° F. that were not crowded and to which fresh food was added regularly.

It will be noticed from table 1 that larvae which had been quiescent for a period of 2 months at 60° or 90° F. are more difficult to kill than active larvae with methyl bromide, hydrocyanic acid, and acrylonitrile. The data indicate that the quiescent larvae held for 2 months at 90° F. may be more difficult to kill with methyl bromide than those held for 2 months at 60° F., whereas with hydrocyanic acid the data indicate a reverse condition.

The possibility exists that penetration of the fumigant into the crevice between the two plastic plates occupied by the quiescent larvae may have been retarded, but it is improbable that this would be a factor since the crevice was approximately 1/16 inch in width and no larva was more than 1 inch from the outside edge of the cell.

RESPONSE TO LOW TEMPERATURES.—Quiescent larvae were held at 60° or 90° F. for 2 and 3 months from the time they entered the crevice in the cell until they were exposed, along with active larvae, for periods of 5, 8, 10 and 12 days to a temperature of 12° F. ±4°. The results obtained indicated the following:

(1) The active larvae were definitely more susceptible than the quiescent larvae at all exposure periods. In all experiments, only one adult was obtained from the active larvae and this at an exposure period of 5 days to 12° F., whereas a considerable number of adults were obtained from quiescent larvae held for 12 days at 12° F. One hundred per cent of the active larvae were killed when held for 12 days at 12° F.

(2) There was some indication that the quiescent larvae held at 60° F. may have been more resistant to exposure to 12° F. than were the quiescent larvae held at 90° F.

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OBITUARIES

William Swift Regan
1884-1959

William S. "Doc" Regan died at his Yakima, Washington home November 11, 1959. Dr. Regan was born August 21, 1884 in Williamsburg, Massachusetts. He attended school there and later received his Ph.D. degree in 1915 at Massachusetts State College. He was Chief Deputy State Nursery Inspector of Massachusetts from 1905–1913; later became Associate Professor at Massachusetts State College in 1921. He joined the staff of Montana State College in 1922. From 1924 until his death, he was entomologist for the California Spray Chemical Company, Niagara Chemical Company and Yakima Valley Spray Company, respectively.

Dr. Regan was one of the first, if not the first, commercial entomologists in Washington. He was highly respected for his keen knowledge of tree fruit entomology by orchardists, fieldmen and other professional colleagues. In his earlier service with the California Spray Chemical Company, he conducted considerable research on evaluating various sprays for control of codling moth and other orchard pests in the Yakima Valley.

Dr. Regan was truly a gentleman. His immaculate dress, articulate speech, and clean-cut appearance was indeed impressive.

He is survived by his wife, Winifred; two daughters, Mrs. Barbara Singer of Seattle and Miss Ann Regan, student at Washington State University; and two sons, William and James, both of Seattle.

Dr. Regan was a member of Gamma Delta Chapter of Kappa Sigma, Rotary, American Association of Economic Entomologists, Fellow of The American Association for the Advancement of Science, Washington State Horticultural Society, and St. Paul Cathedral.

H. S. TELFORD

Frank Atwood Sirrine
1861-1959

Frank Atwood Sirrine who died September 25, 1959, at Riverhead, Long Island, New York, may have been America's oldest living Entomologist. He was in his 97th year. Although inactive in the profession since 1919, he conducted entomological work over a 26-year period prior to 1919 as a staff member of the New York State (Geneva) Agricultural Experiment Station.

Mr. Sirrine shares with Victor H. Lowe (deceased, 1903) the distinction of being the Geneva Station's first Entomologist. Both men reported for duty July 23, 1894, and both were located initially at Jamaica, Long Island, N. Y. Two years later Mr. Lowe was transferred to Geneva leaving Mr. Sirrine in charge of the work on Long Island. The Jamaica Laboratory was closed June 30, 1902. On this same date, although no change in duties apparently was involved, Mr. Sirrine's title was changed from that of Entomologist to Special Agent.

In the years that followed, he became the local authority on insect and plant disease problems, particularly those affecting potatoes and truck crops. Mr. Sirrine always worked closely with his farmer constituency. While this probably was by personal choice, he was active in a period which predated the Extension Service and it was inevitable that grower-service would have claimed most of the time of a local insect and plant disease specialist. Mr. Sirrine resigned his Station post in 1919. This he did to devote full time to the operation of his 245-acre farm at Northville near Riverhead, Long Island, N. Y., which he had owned since about 1902.

Mr. Sirrine was born November 22, 1861, at Newark, Illinois. He taught school in Nebraska to pay for his college training at Iowa State College where in 1893 he received the M.S. degree. He studied there under Professor Herbert Osborn and also served as his assistant for a year following graduation. From Iowa State, Mr. Sirrine came directly to New York.

Mr. Sirrine is the author or co-author of a number of most creditable publications. His earliest professional paper dealt with the distribution and taxonomy of aphids in which he and Professor Osborn described a new species, *Neoprociphilus* (=*Pemphigus*) *attenuatus* Osborn and Sirrine. (Insect Life 5: 235–7. 1893; Iowa Acad. Sci. Proc. 1: 129–131. 1894.) One of his first duties upon coming to New York was to conduct tests on the control of the San Jose scale. His findings represent some of the earliest information on control of this notorious pest. Following these papers, Mr. Sirrine authored a number of Station and other particles on pests of vegetable crops. One of his last writings, done in collaboration with B. B. Fulton, was on the cranberry toad-bug (New York (Geneva) Agric. Expt. Sta. Bull. 377. 1914). This bulletin meets a high professional standard.

From 1919 until his death, Mr. Sirrine engaged in farming on his Northville property. In a district noted agriculturally for its potatoes, ducks, and truck crops, Mr. Sirrine, untypically, was a fruit grower. His was among the largest fruit plantings on Long Island. He grew all kinds of fruit, but his specialty was pears. He is credited with having introduced to the Island the elegant Bosc pear with its russet-coated fruit. It was his custom to spray his Boscs with bordeaux mixture to insure the formation of a uniform russeted skin. He thus turned to an advantage the well-known drawback to the use of this fungicide on smooth-skinned pears or apples.

Although two of his sons joined forces with him recently, Mr. Sirrine was active in his farm operation until the accident which led to his death. On September 23, 1959, he fell and broke a hip. Two days later he died.

Mr. Sirrine was married twice: his first wife died in 1914, his second in 1957. He is survived by four sons, Henry A. and Robert D. Sirrine of Northville, Frank A. Sirrine Jr. of Riverhead, and John H. Mostow (stepson) of Metuchen, N. J.; by two stepdaughters, Mrs. Halsey Hallock, Bronx, N. Y., and Mrs. Ralph Mason, Demarest, N. J.; by a sister, Miss Ida Sirrine, Traer, Iowa, and by 11 grandchildren.

P. J. CHAPMAN
HUGH HUCKETT
MAURIE SEMEL